

Ecology, parasitism and conservation of sparrows in anthropized environments:

Insights from the Working Group on Urban Sparrows



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7th Meeting Working Group on Urban Sparrows
27th-28th October 2023 (Valencia, Spain)

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November, 2025



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Recommended citation:

Bernat-Ponce, S., Mestre, A., Gil-Delgado, J.A. & Bernat-Ponce, E. (2025). Ecology, parasitism and conservation of sparrows in anthropized environments: Insights from the Working Group on Urban Sparrows. *Universitat De València*. <https://doi.org/10.7203/PUV-OA-9788491338444>

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Proofreading and layout: Edgar Bernat-Ponce, Saúl Bernat-Ponce

Illustrations: FreePik, Bioicons

Cover: Wirestock FreePik

Photographs; Tables & Figures: Chapter authors

ISBN (PDF): 978-84-9133-844-4

DOI: <https://doi.org/10.7203/PUV-OA-9788491338444>

Digital edition

“Los gorrones son los niños del aire, la chiquillería de los arrabales, plazas y plazuelas del espacio. Son el pueblo pobre, la masa trabajadora que ha de resolver a diario de un modo heroico el problema de la existencia. Su lucha por existir en la luz, por llenar de píos y revuelos el silencio torvo del mundo, es una lucha alegre, decidida, irrenunciable. Ellos llegan, por conquistar la migaja de pan necesaria, a lugares donde ningún otro pájaro llega. Se les ve en los rincones más apartados. Se les oye en todas partes. Corren todos los riesgos y peligros con la gracia y la seguridad que su infancia perpetua les ha dado.”

"Sparrows are the children of the air, the kids of the suburbs, squares and small squares of space. They are the poor people, the working class that has to heroically solve the problem of existence on a daily basis. Their struggle to exist in the light, to fill the grim silence of the world with chirps and fluttering, is a joyful, determined, unwavering struggle. They go to places where no other bird goes to get the crumbs of bread they need. They can be seen in the most remote corners. They can be heard everywhere. They take all the risks and dangers with the grace and confidence that their perpetual childhood has given them."

El gorrión y el prisionero

Cuento inconcluso. Miguel Hernández (1910-1942)

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PREFACE

We were pleased to welcome you to the **7th Meeting of the Working Group on Urban Sparrows**, held in 2023 (27th-28th Oct), a long-awaited edition after various circumstances and a pandemic delayed our plans.

After four years since our last gathering in Breda (2019), we were finally able to come together again in person, something we considered essential. Even in today's globalized world, where virtual communication offers many advantages, we still believe that face-to-face meetings are irreplaceable. It is in these in-person encounters, often over a coffee, that meaningful collaborations, fresh ideas, and promising projects take root. And the present book is a written reflection of it.

This meeting brought together nearly 30 participants from across Spain, as well as from several European countries, including the Netherlands, Belgium, and Finland. During our time together, we engaged in vibrant discussions, exchanged knowledge, and outlined strategies to continue advancing the conservation of urban sparrows. We intentionally created a space for interaction among experienced researchers, young investigators, and recent graduate future leaders in our field. Built into the program were opportunities for informal dialogue and reflection, including a roundtable discussion that explored the past, present, and future research on the species that unites us. We also took time to enjoy Valencia—its rich gastronomy, cultural heritage, and natural landscapes, which we hope you discovered and appreciated as much as we do.

We express our sincere gratitude to the **Institut Cavanilles de Biodiversitat i Biologia Evolutiva**, its director **Joaquín Baixeras**, and its secretary **Francesc Mesquita**, for their financial support and for providing an ideal setting for the meeting. We are also grateful to the **Institut Cavanilles de Biodiversitat i Biologia Evolutiva** for funding this open access book, published by **Publicacions de la Universitat de València**, which makes the outcomes of the meeting widely accessible to the scientific community and the general public.

Finally, and most importantly, thank you to all participants for your interest, enthusiasm, and engagement with this book.

Edgar Bernat-Ponce

Organizer and Member of the Scientific Committee of the 7th WGUS Meeting

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BOOK OF ABSTRACTS
“WORKING GROUP ON URBAN SPARROWS”
2023 MEETING
VALENCIA (SPAIN)

Only abstracts from the WGUS 2023 Meeting that are not represented by a full chapter in this book have been included.

- **Conservation Strategies for House Sparrow Conservation**

Author: Mohammed Dilawar

Affiliation: Nature Forever Society

Presenter: Mohammed Dilawar (dilawarmohammed@gmail.com)

Abstract: I would like to present that how we were able to create awareness, large scale habitats, increase people's participation and advocacy to save House Sparrows and other common flora and fauna In Indian and other parts of the world. How we used traditional and modern social media to create a peoples moment to save House Sparrows and how functional conservation along with functional conservation initiatives like World Sparrow Day (www.worldsparrowday.org) getting the Sparrow Declared as the State Bird of Delhi, Adopt Nest boxes and bird feeders and Getting Guinness Book of World Records for Largest Bird Feeder Distribution helped in Large Scale Conservation and Awareness of House Sparrows in India.

Keywords: House Sparrow, World Sparrow Day, conservation, habitat creation, functional conservation

- **North American House Sparrows: Tales from the invasive populations of Mexico and the USA**

Authors: Ian MacGregor-Fors¹, Javier Quesada², Pamela J Yeh³

Affiliations: ¹University of Helsinki, ²Museu de Ciències Naturals de Barcelona, ³UCLA

Presenter: Ian MacGregor-Fors (ian.macgregor@helsinki.fi)

Abstract: The North American invasion of the House Sparrow is one of the best studied. In recent years, evidence has shown interesting ecological and behavioral differences between some of their populations when contrasted with each other, and also when compared to European ones. In this talk we summarize the findings of projects we have lead related to the North American invasion of House Sparrows, spanning from their distribution across urbanization gradients, their densities, ecology, and behavior. Lessons learned from these invasive populations could shed some light in understanding the global distribution and population trends of the species.

Keywords: Mexico, USA, Spain, invasive, behavior

- **Does urban parks management in a large city affect parasitism in the Tree Sparrow?**

Authors: Eva Banda¹, Aida Vega¹, Beatriz Martínez-Miranzo¹, Ana López¹, Hernández. S^{2,3}, Yabsley, M.^{2,3}, José I. Aguirre¹

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Presenter: Eva Banda (ebanda@ucm.es)

Abstract: The management strategies applied to urban parks have a significant influence on the composition of bird communities and parasitism rates, which can, in turn, affect population conservation. The Eurasian Tree Sparrow is an exemplary species that has successfully adapted to urban life due to the deterioration of its original habitat. However, this adaptability comes with challenges, notably in relation to parasitism, where it exhibits higher prevalence rates compared to House Sparrows. To investigate the impact of different management practices on parasitism in urban parks, researchers studied the degree of parasitism, including blood and intestinal parasites, in Tree Sparrows across various green areas in the city of Madrid. Notably, green areas with distinct management approaches showed variations in parasitism levels, possibly linked to their treatment of water bodies. This not only affects avian species but could also have implications for other animals, including humans. In conclusion, effective management of urban parks can significantly contribute to biodiversity conservation. Understanding the relationships between management practices, bird communities, and parasitism prevalence is crucial for developing sustainable strategies to promote urban biodiversity and protect both wildlife and human populations.

Keywords: parasitism, Tree Sparrow, management strategies, urban parks

- **PASSERCAT: Ecology of a declining species, the House Sparrow in Catalonia**

Authors: Javier Quesada¹, Ian MacGregor-Fors², Santiago Guallar¹, Rocío Pérez-Portela³, Joan Calderon¹, Johan Espunyes⁴, Maria Puig Ribas⁴

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Presenter: Javier Quesada (jquesadal@bcn.cat)

Abstract: This contribution tackles the conservation biology of the House Sparrow using monitoring, genetic and behavioral data to understand the decline of urban populations of this species in Catalonia (Spain) since 2012. House Sparrow declines are steeper in urban areas than other habitats. Count data suggest that the presence of House Sparrows in urban areas is positively related to the age of the building (availability of nesting holes), distance to orchards and presence of litter. Nest-box supply is a good conservation tool to reinforce breeding populations but not to establish new colonies. Long term survival is similar in urban and rural populations. The prevalence of *Plasmodium* was relatively low compared with that of other European populations. Genetic (allelic) richness has not changed in the last century but populations are different from the original ones, suggesting genetic drift processes. Lack of relationship between low allelic richness and negative population trends suggests that inbreeding processes are not a problem for Catalan populations of the House Sparrow yet. Altogether, these results show a similar decreasing pattern of other European populations. A wider scale approach is needed to disentangle how the drivers of the species decline vary at different geographic scales from a European perspective.

Keywords: Catalonia, conservation biology, House Sparrow, population decline, urban sparrows

- **Seasonal Survival Patterns in House Sparrows (*Passer domesticus*) in Urban Environments: a study case in a Mediterranean city**

Authors: Edgar Bernat-Ponce^{1,2}, José A. Gil-Delgado¹, Germán M. López-Iborra³

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Presenter: Edgar Bernat-Ponce (edgar.bernat@universidadeuropea.es)

Abstract: Seasonality, driven by environmental changes, profoundly affects various biological processes in birds, including reproduction, growth, migration, and survival. The urban environment, expanding rapidly due to human population growth, presents challenges for urban birds. House Sparrows, a classic urban exploiter, face long-term population declines with multicausal factors. Limited data on adult and juvenile survival in Mediterranean countries like Spain adds to the complexity. The seasonal survival pattern of a House Sparrow population was studied in an urban ecosystem, a city park of Alcoy Eastern Spain. Ringing was carried out between April 2017 and September 2019 while resighting surveys were carried out until summer 2020. In total, 195 House Sparrows were captured and colour-marked during the four year study period and 145 (74.36%) were recaptured during the survey. Best models showed no differences in seasonal survival between sexes but a reduced survival in summer (end of breeding season). Annual survival shows a decline trend with time that overlaps with the survival seasonal cycle. The preliminary results show that House Sparrow in urban areas presents a negative trend in survival with time and that survival is lower in summer in adults. Our study suggests that urban sparrow decline might be linked to negative trends in survival and that breeding season is very demanding for individuals.

Keywords: bird ringing, capture-recapture, colour ringing, MARK program, seasonality.

- **The House Sparrow *Passer domesticus* in the Netherlands: recent increase, what's going on?**

Author: Jan Schoppers

Affiliations: Project lead Monitoring Urban Species MUS, Sovon Bird Research Netherlands.

Presenter: Jan Schoppers (Jan.Schoppers@sovon.nl)

The House Sparrow is on the European Red List as 'Least Concern' (Birdlife International 2023). The species is Red Listed (near threatened) in the Netherlands. In the eighties and nineties of the previous century, the Dutch breeding population halved. Around the turn of the century it stabilized and a few years later it actually set in a recovery: the national numbers increased 2011-22 by about 2% each year. This recovery only takes place in the countryside, especially in the High Netherlands (above sea level, sand). In the urban habitat the numbers are stabilized (MUS, 2007-22). This large local variation in urban House Sparrow-trends is partly determined by size and manner of construction of new buildings renovation projects and the way with 'green in the neighborhood' is handled by the municipality and residents. Important factors here include offering alternative nesting facilities during insulation work, study its effectiveness and the management of public green areas and the design of gardens (hedges, shrubs).

Threats and chances

Large-scale post-insulation and renovation projects for the energy transition mean that roofs are adapted to the current time. Nesting sites of House Sparrow, Swift *Apus apus* can be destroyed and can have a huge impact on the populations (2,5 million houses!). Sovon conducted research in 2022 and 2023 at 15 locations in the Netherlands into the occupancy of alternative nest sites by the House Sparrow compared to natural nesting sites. The results are reported in September and I will give you a view during the meeting. Thereby 980.000 new houses have to be built in the coming decade.

Year of the House Sparrow in 2024

Next year Sovon and Birdlife the Netherlands called out the Year of the House Sparrow. We will focus on the next subjects:

- Desk study relationship trends/densities and environmental variables
- Extra effort on research on breeding success of the House Sparrow

- Census nesting sites and success and the opportunity for using the Dutch All year round garden count
- Campaign within the annual Garden Bird Count
- Analysis Ring-MUS data by Vogeltrekstation (The Dutch Ringing Centre) & data research RAS project René Oosterhuis (and Cees Heij)

Keywords: little increase, countryside, insulation houses, alternative nest sites, 2024 Year of the House Sparrow

- **Plastic debris in nests of Tree Sparrow (*Passer montanus*)**

Authors: Álvaro Luna¹, Estel Blanch²

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Presenter: Estel Blanch (estelblanchojea@gmail.com)

Abstract: Plastic pollution has become a novel threat that affects global biodiversity. In the present work, we have surveyed nest boxes of the Eurasian Tree Sparrow (*Passer montanus*) and Parids in 5 historical parks of Madrid, Spain. Our results confirm the presence of plastic in a large percentage of Tree Sparrow nests. The collected plastic has a domestic origin, related to the recreational use of the parks and the garbage of the surrounding streets. In fact, we also detect how the nests closest to the edge of the parks with the streets have a greater amount of plastic. In addition, we have detected a selection pattern, as Tree Sparrows mostly use transparent film-type plastics, while they don't use many plastics of other colours or fibres, as Parids do. Last, Tree Sparrows seem to use plastic debris with a structural function, unlike other species, which seem to use plastic fragments for ornamentation. This is one of the first studies to analyse the presence of plastic in urban birds and also in Tree Sparrows, showing that it may be more widespread than previously thought. Future research is needed to establish comparisons with other populations and also with the House Sparrow across its European range.

Keywords: House Sparrow, plastic pollution, urban, park, anthropogenic material

- **A cunning Eurasian Tree Sparrow**

Author: Iris Solís Hernanz, Carla Esteban, Elena Álvarez, Emilio Barba

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Presenter: Iris Solís Hernanz (soheri@alumni.uv.es)

Abstract: Competitive relationships between hole-nesting passerine bird species can lead to mixed broods when two or more species lay their eggs in the same nest. On 5 May 2023, during routine inspection of nest boxes in orange plantations in Sagunto (eastern Spain), we found a Eurasian Tree Sparrow (*Passer montanus*) egg dumped among nine Great Tit (*Parus major*) eggs. From this clutch, six Great Tits and the Eurasian Tree Sparrow hatched, and four Great Tits and the Sparrow fledged. Using a video camera inside the nest box, we observed that only Great Tits fed the nestlings, bringing caterpillars, moths and spiders, a typical Great Tit nestling diet in this habitat. A male and a female Great Tit were later trapped at the nest box while feeding nestlings. As far as we are aware on Great Tit/Eurasian Tree Sparrow relationships, Great Tit eggs had been found in Eurasian Tree Sparrow nests, though none of them seem to have produced Great Tit fledglings. However, this is the first case where the Great Tit acts as host and the Eurasian Tree Sparrow as intruder and, moreover, the sparrow egg hatched, and the chick successfully fledged with its half-sibling Great Tits.

Keywords: mixed broods, mixed clutches, nestling diet, *Parus major*, *Passer montanus*

- **Long-term population trends of House Sparrow and Eurasian Tree Sparrow in Spain**

Authors: Elena Ramos-Elvira, Eva Banda, Juan Arizaga, David Martín, José I. Aguirre

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Presenter: Elena Ramos-Elvira (eleram04@ucm.es)

Abstract: Urban areas are constantly increasing, which can cause an effect in bird populations since human activities lead to nature alterations. Populations of House Sparrow (*Passer domesticus*) and Eurasian Tree Sparrow (*Passer montanus*) have been decreasing in Spain according to the latest national censuses in Spain. In this study, we tried to assess the population trend over more than two decades using ringing data from Spanish constant effort sites, as well as to determine the population and breeding success proxy in relation to habitat composition at landscape level. We analysed the data and confirmed the decreasing trend in the two species. However, Eurasian Tree Sparrow showed signs of increasing presence in urban areas. Furthermore, the productivity remained stable over sampling sites and years, meaning that the causes of the decreasing populations are affecting both adult and juvenile individuals.

Keywords: habitat composition, productivity, urbanization, constant effort sites

- **Are music festivals fun for everyone? The Tree Sparrow (*Passer montanus*) as an indicator of the impact of crowded activities in Madrid.**

Authors: Beatriz Martínez-Miranzo, Eva Banda, José I. Aguirre

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Presenter: Beatriz Martínez-Miranzo (bmartinez@ucm.es)

Abstract: Human activities have an impact on biodiversity and many studies have tried to identify which of these activities have a greater or lesser impact on it. Few studies evaluate the impact on biodiversity of recreational activities, which are held year after year in the same place. We present the first evaluation of the effect of the Festival Las Noches del Botánico on urban biodiversity in Madrid. We used occupancy, productivity and mortality data from 170 nest boxes of Tree sparrow (*Passer montanus*) distributed around the Complutense University of Madrid between 2012 and 2022 (5 years without event and 5 years with event). In the same period, we calculated three biodiversity indices (q0: Species richness; q1: Effective species richness and q2: Dominant species richness) using data from two ringing stations in the study area. There are significant differences in dominant species richness (q2) (i.e; Tree Sparrow) with a significant decrease in their relative density (= capture rate) in the years with an event. Although the rate of nest box occupancy by the Tree Sparrow remained constant, an overall decrease in productivity and an increase in mortality rate were observed in the years of the event. This study highlights the importance of long-term monitoring programs as a tool for correct management and conservation of biodiversity.

Keywords: biodiversity, urban area, urban management, ringing, nest boxes

- **How does the type of urbanization affect parasitism and the immune status of the House Sparrow in a large city and its peri-urban areas?**

Authors: Eva Banda¹, Selene Ledesma, Aida Vega¹, Beatriz Matínez-Miranzo¹, Sonia Hernández³, Michael Yabsley^{2,3}, José I. Aguirre¹

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Presenter: Eva Banda (ebanda@ucm.es)

Abstract: The House Sparrow (*Passer domesticus*) is an anthropophilic bird, with a worrying global decline in its populations in both urban and peri-urban environments. Parasitism represents one of the greatest challenges for the immune balance of vertebrates, so its study is important since it can have consequences on populations and affect their possible conservation measures. We have studied the degree of parasitism (prevalence and abundance of blood and intestinal parasites) of different urban and peri-urban populations of house sparrows in the city of Madrid, their immune status related to their body condition, and the chronic stress to which they were exposed. Differences have been found in some aspects of parasitism depending on the type of urbanization associated with the immunological and body condition.

Keywords: parasitism, immune status, body condition, house sparrow, urbanization

- **Actions, alliances and social awareness for urban sparrow's conservation**

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Affiliation: Sociedad Española de Ornitología (SEO/BirdLife)

Presenter: Anna Valentín Benzal (avalentin@seo.org)

Abstract: Data collected from SEO/BirdLife Citizen Science program (common bird monitoring program- SACRE) shows that House Sparrow populations have decreased in the last 25 years in Spain in parallel with the European population. Although we have observed some recovery in the past three years, the urban sparrow population has decreased by an average between years of 0.8% since 1998, being more pronounced in urban centers than in rural areas.

To tackle this situation and being aware of the importance of human sensitization of the species for them to thrive, we have produced several reports on the urban sparrow and other common species conservation status and threats. We also generated alliances with the Environmental Ministry to span the improvement of shelter and nest sites in Spanish cities through the "Technical Guide to conserve and promote biodiversity in new construction and rehabilitation".

Finally, we have developed several social awareness campaigns, such as "Aves de Barrio", "SOS Nidos" or the House Sparrow Day, and documents to enhance biodiversity on gardens and balconies.

Keywords: Social Awareness, urban birds, buildings, urban fauna.

Book Chapters

Ecology, parasitism and conservation of sparrows in anthropized environments:

Insights from the Working Group on Urban Sparrows

Anthropization and avian malaria infection effects on fatty acids composition and oxidative stress of wild House Sparrows

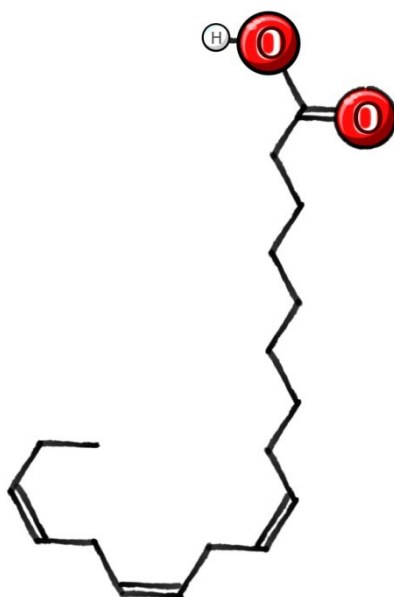
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Anthropization and avian malaria infection effects on fatty acids composition and oxidative stress of wild House Sparrows

Jéssica JIMÉNEZ-PEÑUELA

ABSTRACT

Low availability of good quality food, pollutants and other stressors may prevent urban birds from fulfilling their physiological requirements, affecting their responses against pathogens. This study identified the impact of habitat anthropization and avian malaria infections on the physiology of wild juvenile House Sparrows (*Passer domesticus*), an urban-dwelling passerine which populations are declining. The infection by *Plasmodium*, *Haemoproteus* and *Leucocytozoon*, the fatty acids abundance and composition, the oxidative lipid damage (measured as TBARS) as well as the activity of antioxidant enzymes (GPx, SOD and GR) were measured in blood from birds captured in urban, rural, and natural habitats from southern Spain. Birds from urban habitats had higher levels of fatty acids and higher oxidative lipid damage than those from rural habitats. Antioxidant enzymatic activity was negatively associated with bird's body condition. *Plasmodium* infected juveniles showed lower proportion of ω -6 PUFAs, higher proportion of ω -3 PUFAs and lower activity of antioxidant enzymes than uninfected ones. Moreover, *Plasmodium*-infected birds showed a lower ω -6/ ω -3 ratio and tended to have a higher proportion of ω -3 fatty acids compared to uninfected birds, but only in natural habitats. These results suggest that birds exposed to two different sources of stress—urbanization and parasite infection—may experience more severe negative effects, which can impact various physiological parameters and impose greater energetic or physiological costs.

KEYWORDS

Infectious diseases, *Passer domesticus*, physiology, urbanization, wildlife

INTRODUCTION

In less than a century human population has almost tripled. The intensification of agriculture, animal husbandry, and the expansion of cities have provoked important landscape transformations associated with biodiversity loss and linked to an alteration in the interactions between organisms, including disease transmission (Keesing et al., 2010). Cities provide highly predictable and stable resources (Sumasgutner et al., 2014). However, low quality of food, the presence of pollutants and other environmental stressors derived from the human activities are major factors negatively affecting wildlife in urban areas (Delhayé et al., 2018; Salmón et al., 2018b). Food of anthropogenic origin is usually rich in carbohydrates and fats but poor in proteins (Heiss et al., 2009). The substitution of suitable preys like protein-rich invertebrates by human-derived food negatively affects nesting success, fledgling growth, immunocompetence and survival (Peach et al., 2015). Differences in fatty acid composition can be observed in birds from urban and rural areas (Andersson et al., 2015; Isaksson et al., 2017), mainly in the ω -6 and ω -3 polyunsaturated fatty acids (PUFAs), which can modulate both cellular and humoral immune responses by affecting the production of lymphocytes, heterophils and splenocytes, as well as IgM and IgG antibodies (Alagawany et al., 2019). Additionally, ω -6 PUFAs are metabolized to pro-inflammatory prostaglandins, while the ω -3 PUFAs are metabolized into anti-inflammatory agents, so an alteration in the ω -6/ ω -3 ratio has been associated with changes in immune responses and in oxidative stress (Hulbert et al., 2005; Simopoulos, 2011).

Oxidative stress occurs when the balance between reactive oxygen substances (ROS) and antioxidants is lost, thus causing oxidative damage to biomolecules including proteins, lipids and DNA (Li et al., 2016). ROS are either derived from an internal source, as by-products of basal metabolism and inflammatory responses to fight external aggressions (Costantini, 2019; Sorci & Faivre, 2009), or from a synthetic external source such as anthropic activities. Indeed, birds living in urban habitats are exposed to toxic chemicals, heavy-metal particles and other pollutants such as NO_x, SO₂ and O₃, generated by the burning of fossil fuels producing greater oxidative stress and oxidative damage on

organisms (Herrera-Dueñas et al., 2014; Salmón et al., 2018a). Indeed, Great Tits from urban habitats have higher levels of expression of genes related to immune, inflammatory and antioxidant responses than those birds breeding in natural habitats (Watson et al., 2017). Producing inflammatory and immune responses is energetically costly, so the capacity of the host to effectively fight off infections are determined by their current body condition and nutritional status (Cornet et al., 2014; Delhayé et al., 2018; Navarro et al., 2003). Considering that both may be altered in more anthropized environments, a special trade-off between producing inflammatory and immune responses may occur in cities. Both correlative and experimental studies indicate that malaria-related parasites are common sources of physiological stress (Isaksson, 2015). Avian malaria parasites of the genus *Plasmodium* and the related *Haemoproteus* and *Leucocytozoon* are widespread vector-borne parasites transmitted by insect vectors (Atkinson et al., 1991; Valkiūnas, 2005).

Factors such as nutritional constraints, large-scale intensive animal husbandry and agriculture, pollution, pesticides, and infectious diseases like avian malaria, have been suggested as potential causes for House Sparrow (*Passer domesticus*) population decline across Europe, especially in cities (De Laet and Summers-Smith, 2007; Hanson et al., 2020). This bird is a widespread, abundant, sedentary and urban exploiter passerine historically known to thrive in areas with human activities and settlements, living in direct contact with humans for centuries while taking advantage of anthropogenic food resources and cavities in buildings for nesting (Hanson et al., 2020). This species has a low dispersal rate and a small home range radius of 1000–2000 m, and only around 10% of juveniles disperse more than 2000 m from their natal locality (Ranke et al., 2024). Additionally, House Sparrows are frequently infected by different blood haemosporidians including, at least, 40 genetically different lineages of *Plasmodium*, 16 lineages of *Haemoproteus* and 12 lineages of *Leucocytozoon* (according to MalAvi database accessed 30 April 2024). These characteristics make the House Sparrow an excellent study model for assessing the joint impact of urbanization and parasite infections on individual physiological traits (Hanson et al., 2020). This work aims to compile the potential synergic effect of urbanization and parasite infection on the House Sparrow health reflected in two complimentary physiological

variables such as fatty acids composition and oxidative stress status. Most part of the information presented here comes from previously published scientific articles (Jiménez-Peñuela et al., 2023, 2022).

MATERIAL AND METHODS

Study design and bird sampling

Birds were sampled in the province of Huelva, Seville and Cadiz, southern Spain, at 45 localities grouped into 15 geographically close triplets consisting of one urban, one rural and one natural habitat reflecting a gradient of urbanization. For further information on the sampling design, see Ferraguti et al. (2016). From July to October 2013 wild House Sparrows were caught using mist-nets and a bird call playback recorder. Each individual was ringed with a numbered metal ring, and sexed and aged according to their plumage characteristics and skull ossification when possible (Svensson, 2009). Birds were classified as juveniles, adults or of unknown age. Samples from juveniles (younger than one year; EURING code 3) were exclusively analysed due to their relevance to provide information about the environmental conditions and parasite circulation in the area occurring during spring and summer of the sampling year (Valkiūnas, 2005). Bird body mass was measured with an electronic balance (digital scale to the nearest 0.1g), and wing length was measured with a metal ruler (to the nearest 0.5 mm). Both variables were used to estimate individual body condition according to Peig and Green (2009). A blood sample was extracted from the jugular vein using a sterile syringe and never exceeding 1% of the bird's body mass. Blood samples were stored in Eppendorf tubes maintained in cold boxes during field work, and subsequently kept for less than 24 hours at 4°C until centrifugation for 10 min at 1700g (4000 rpm) to separate serum and cellular fractions. The cellular fractions were frozen at -20°C and serum at -80°C until further molecular and biochemical analysis. Birds were released at the place of capture without any apparent damage.

Molecular analyses

Genomic DNA was extracted from the blood cellular fraction using the Maxwell®16 LEV System Research. Sex determination was performed by plumage characteristics, or genetically following the protocols by Griffiths et al. (1998). The infection status and the identity of the parasite genera were assessed following Hellgren et al. (2004), and sequenced by MacroGen. Sequences were edited using the software Sequencher™ v 4.9; the parasites genera were identified by comparison with sequences deposited in GenBank and MalAvi databases (Bensch et al., 2009).

Biochemical Analyses

The FA analyses (Jiménez-Peñuela et al., 2022) were run in 165 individuals of both sexes, uninfected or infected by *Plasmodium*, captured in 15 of the 45 sampled localities. Fatty acids were extracted from serum samples following the protocol described in Andersson et al. (2015), analysed by Gas Chromatography/Mass Spectrometry (GC/MS), identified using their mass spectra and retention time, and quantified through their peak area. The oxidative stress analyses (Jiménez-Peñuela et al., 2023) were done in the blood cellular fraction of 688 juvenile males from 42 localities sampled (one triplet was excluded given the low number of juvenile male House Sparrows captured at one locality). The oxidative damage to lipids was quantified through thiobarbituric acid reactive substances (TBARS, n=600). The antioxidant capacity of birds (n=488) was measured through the activity of the superoxide dismutase (SOD), the glutathione peroxidase (GPx), and the glutathione reductase (GR), referring to the total protein content expressed in mg/ml. All methods were adapted to the Cobas Integra 400. A Principal components analysis (PCA) was performed to summarize the antioxidant activity of SOD, GPx and GR into two principal components. The one reported here reflects the relative antioxidant activity of GR (positive) comparing to the activity of SOD and GPx (negative), hereafter known as antioxidant enzymatic activity.

Statistical analysis

The statistical methods used for obtaining the main results reported here are fully explained in Jiménez-Peñuela et al. (2023, 2022). Briefly, Linear Mixed-Effects Models (LMM) fitted by maximum likelihood were used to test the effects of habitat category (urban, rural, and natural), malaria infection status (infected and uninfected) and their interaction. Hour, day of capture, and bird body mass or condition were also considered. Locality nested in triplet were included as random factors to account for the geographical stratification of the sampling design. The differences between levels of a category were tested with Tukey's post-hoc tests, and the interaction between two factors with package *phia*. The sum of squares was calculated using an ANOVA type III, while the conditional (R^2_c) and the marginal (R^2_m) variance explained were determined by a pseudo- R^2 . The normality of continuous variables and the residuals were tested with *qq*-plots. All statistical analyses were conducted in R (version 4.0.4 "Lost Library Book"; R Foundation for Statistical Computing 2021). Additionally, here we tested whether TBARS levels differed between zones when controlling by the PUFAs proportion, including in the analyses the bird weight and *Plasmodium* infection status. All graphs were created with *ggplot*.

RESULTS

The total FA concentration was significantly associated with the habitat category, with urban individuals showing higher values than rural birds; similar values were found in birds from urban and natural habitats, and in birds from rural and natural habitats (Jiménez-Peñuela et al., 2022). The oxidative damage to lipids (TBARS) was marginally linked to habitat category, with rural birds showing lower TBARS levels than urban ones; no differences were found for the rest of comparisons (Fig. 1-A, (Jiménez-Peñuela et al., 2023)). In a selection of 55 individuals on which both fatty acids and oxidative stress were analysed (Table 1), we found that TBARS levels were not influenced by total PUFAs levels, although they significantly varied between habitats. Urban birds showed higher TBARS levels compared to rural (Est=0.22, S.E.=0.09, z value=2.4; p =0.04), and rural had slightly lower TBARS levels than natural birds (Est=-0.2, S.E.=0.08, z value=-2.34; p =0.05). No

differences were found between urban and natural habitats (Est=0.02, S.E.=0.08, z value=0.2; $p=0.98$; Fig. 1-B). Lastly, the relationship between TBARS and PUFAs levels marginally varied between zones, with urban and natural habitats showing a positive correlation but rural habitats showing negative correlation (Fig. 1-C).

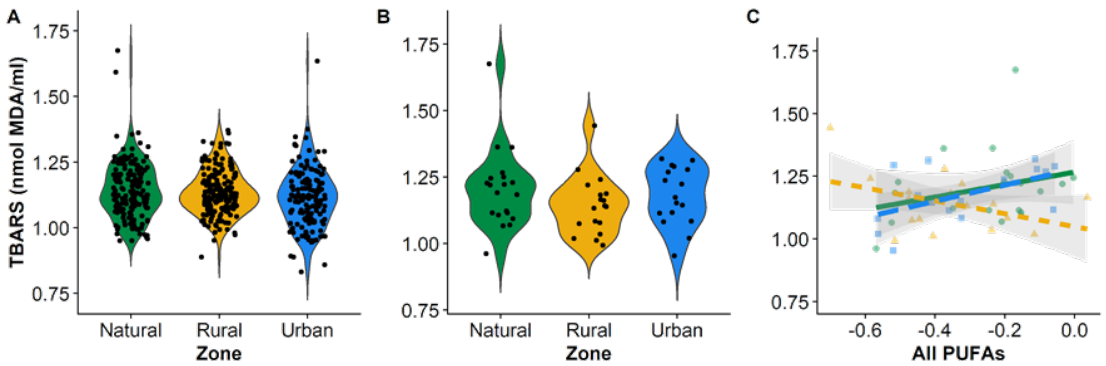


Figure 1. (A) TBARS levels of all birds analysed in oxidative stress analyses ($n = 600$), (B) TBARS levels of birds included in both oxidative and fatty acids analyses ($n=55$), and (C) the relationship between TBARS levels and PUFAs levels of birds included in both analyses ($n=55$).

Table 1. Results of the Linear Mixed Models (LMM) analysing the effect of bird's body mass, *Plasmodium* infection status (infected and uninfected birds), habitat (urban, rural, and natural), the total Polyunsaturated Fatty Acids (PUFAs), and the interaction between habitat and PUFAs levels on the oxidative damage to lipids (TBARS). Significant relationships ($p \leq 0.05$) are highlighted in bold. Conditional (and marginal) R^2 are shown. Est = Estimate; S.E.= Standard Error; χ^2 = chisq; d.f. = degree of freedom; p = p-value; ^a = Reference category.

	Estimate $\pm$ S.E.	χ^2	d.f.	p
Intercept	1.1 $\pm$ 0.22	24.73	1	<0.001
Body mass	0.006 $\pm$ 0.008	0.53	1	0.465
<i>Plasmodium</i>:0	0.00 ^a	0.08	1	0.772
<i>Plasmodium</i>:1	0.01 $\pm$ 0.04			
Habitat:Natural	0.00 ^a	6.94	2	0.031
Habitat:Rural	-0.2 $\pm$ 0.09			
Habitat:Urban	0.01 $\pm$ 0.08			
ALL PUFAs	0.23 $\pm$ 0.18	1.65	1	0.199
HabitatNatural:ALL PUFAs	0.00 ^a	5.22	2	0.074
PUFAs				
HabitatRural:ALL PUFAs	-0.44 $\pm$ 0.25			
HabitatUrban:ALL PUFAs	0.09 $\pm$ 0.23			
R^2c (R^2m)			0.21 (0.17)	

Compared to uninfected, *Plasmodium* infected birds had a significantly lower proportion of ω -6 PUFAs, higher proportion of ω -3 PUFAs (Jiménez-Peñuela et al., 2022), and lower antioxidant enzyme activity; birds infected by *Haemoproteus* showed significantly higher TBARS levels than uninfected ones (Jiménez-Peñuela et al., 2023). Additionally, the ω -6/ ω -3 ratio differed significantly with the infection status of birds, with the interaction between the infection status and habitat category being also significant. *Plasmodium* infected birds had lower ω -6/ ω -3 ratio levels than uninfected individuals in natural habitats, but there were no significant differences in birds from rural or urban habitats (Fig. 2-A). In addition, we found a marginally significant effect of the interaction between infection status and habitat on the proportion of ω -3 PUFA, with infected birds

showing higher proportion of ω -3 PUFA than uninfected birds in natural habitats, while non-significant differences were found for the other habitat types (Fig. 2-B).

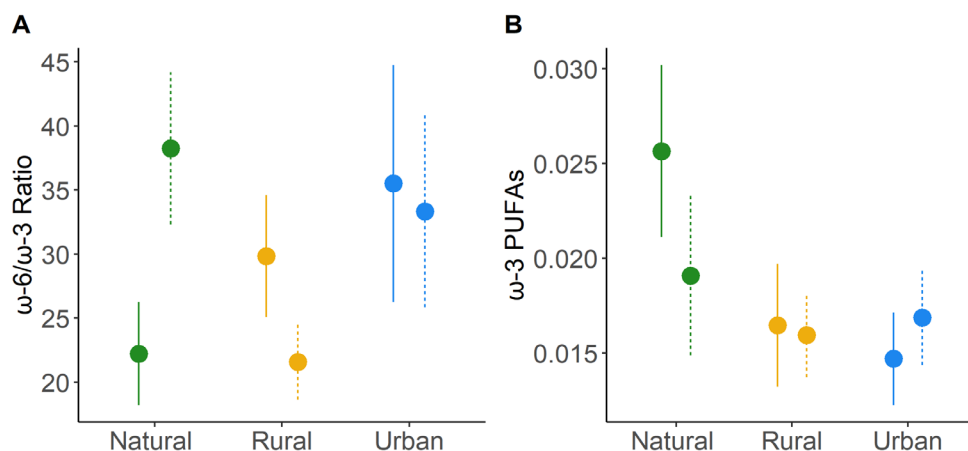


Figure 2. (A) Mean $\pm$ standard error of the ratio omega-6 (ω -6) and omega-3 (ω -3), and (B) mean $\pm$ standard error of ω -3 PUFAs proportion in *Plasmodium* infected (solid line) and uninfected birds (dashed line) from natural (green), rural (yellow), and urban (blue) habitats. Figure adapted from Jiménez-Peñuela et al. (2022).

The body condition was negatively associated with the antioxidant enzymatic activity (Jiménez-Peñuela et al., 2023). That is, birds with higher condition reflected lower GR activity against higher GPx and SOD activity (Fig. 3-A). Additionally, the relationship between TBARS and body condition was significantly different for *Haemoproteus* infected and uninfected birds, being negative for infected birds but positive for uninfected ones (Fig. 3-B). Similarly, the relationship between TBARS and body condition was marginally different among habitats, being positive in natural and rural habitats but negative in urban habitats (Fig. 3-C).

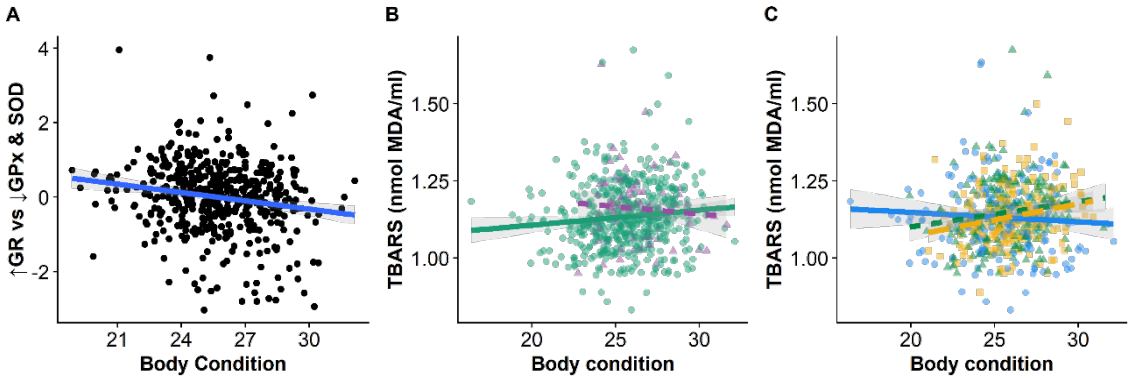


Figure 3. Relationship between birds' body condition and (A) enzymatic antioxidant activity*, (B) TBARS levels of *Haemoproteus* infected (purple dotted line and triangles) and uninfected birds (green straight line and circles), and (C) TBARS levels of birds from natural (green dotted line and triangles), rural (yellow punctuated line and squares) and urban (blue straight line and circles) habitats. * Relative antioxidant activity of Glutathione reductase (GR) compared to the activity of Glutathione peroxidase (GPx) and Superoxide dismutase (SOD). The 95% confidence level interval is shown in grey. Figure adapted from Jiménez-Peñuela et al. (2023).

DISCUSSION

A summary is provided on how habitat anthropization, avian malaria infection and its interaction affect physiological variables of wild House Sparrows from an urbanization gradient, accentuating the relationship between these factors (i.e., TBARS and PUFAs).

The effects of anthropization on birds' physiology

The higher quantity of fatty acids in bird's blood from urban habitats suggests that more fat-rich food is consumed in urban than in rural habitats (Heiss et al., 2009). Although this ensures short-term survival of birds, a nutritionally suboptimal diet negatively affects their growth, body condition, immunocompetence and oxidative stress status (Bernat-Ponce et al., 2023; Houston et al., 2007). Moreover, juveniles from urban habitats had higher levels of oxidative damage to lipids (measured by TBARS) than birds from rural habitats. Urbanization levels highly correlate with NO_x levels, one of the main pro-oxidants and stressors substances in urban areas (Salmón et al., 2018a). Other studies have found

higher oxidative damage to lipids (reflected in TBARS levels) in birds from urban habitats than in birds from less anthropized habitats (Amri et al., 2017). The oxidative damage to lipids is positively associated with blood concentrations of plasma triglycerides (the main form of storage and transport of PUFA) in different bird species and ages (Pérez-Rodríguez et al., 2015). Thus, a subsample of birds ($n=55$) included in both oxidative stress and fatty acids analyses were reanalysed here to check whether differences in TBARS levels between habitats were influenced by PUFAs levels. No significant differences were found, suggesting that the marginal effect found for TBARS levels in Jiménez-Peñuela et al. (2023) is not biased by FA composition. The relationship between TBARS and PUFAs levels was positive for birds from urban and natural habitats, as predicted and proved by Pérez-Rodríguez et al. (2015). Conversely, this relationship was negative for birds from rural habitats. These areas are commonly interpreted as intermediate habitats when studying urbanization. Even so, food availability, contaminants such as pesticides, and other stressors that birds have to face here (de Montaigne and Goulson, 2022) could as well alter physiological biomarkers and introduce noise to urbanization studies. Deeply inspecting these mid-altered habitats could help future studies.

The effects of avian malaria infection and habitat on birds' physiology

During infection, ω -6 PUFA are metabolized to pro-inflammatory, and ω -3 to anti-inflammatory prostaglandins (Alagawany et al., 2019). Thus, in infected birds, lower ω -6 could imply either a reduction in the cost of infection or an increment of susceptibility to infection (Sorci and Faivre, 2009). By contrast, higher ω -3 could be beneficial for responding to infections without increasing the level of inflammation. Infected birds could be preferentially consuming or mobilizing ω -3. This probably happens in natural habitats but less so in more anthropized areas due to the differential availability of ω -3 PUFA (Andersson et al., 2015; Isaksson et al., 2017). Indeed, it is likely the reason why differences in ω -6/ ω -3 ratio were only found in infected birds from natural habitats. Under limited resources, a trade-off may exist between developing antioxidant and immunological responses (Morales et al., 2006). Furthermore, birds exposed to two different sources of stress – urbanization and parasite infection – may suffer more severe harmful effects.

Alternatively, it is also possible that birds from more anthropized environments already metabolize the ω -3 PUFAs for anti-inflammatory components for other reasons (e.g. against environmental stressors such as chemicals, pollutants, excessive noise, artificial light or heavy metal particles), thereby leaving fewer circulating ω -3 PUFAs to be used for other important functions. Moreover, *Plasmodium* infections may reduce the capacity of birds to display the antioxidant responses involved in the catalytic reactions to reduce ROS (Isaksson, 2015). Indeed, similarly to Jiménez-Peñuela et al. (2023), van de Crommenacker et al. (2012) also detected low antioxidant capacities in birds infected with blood parasites. If a pro-inflammatory response is not correctly counteracted, it can lead to an increment in oxidative stress (Isaksson et al., 2017), as shown here in the case of *Haemoproteus* infected birds. Even though the results reported here do not allow to identify the causality of the associations, they may reflect that if infected birds have a reduced antioxidant activity, only those with moderate levels of pro-inflammatory FAs are likely to counteract infections.

The role of body condition in shaping the effects of anthropization and infection status on birds' physiology

Juvenile birds with higher body condition displayed more GPx and SOD activity but less GR activity. The GPx and SOD interact directly with molecules acting as ROS; thus, higher activity implies lower possibility of oxidative damage and, therefore, to lower levels of TBARS. Indeed, our results showed that birds' body condition was positively related to TBARS in rural and natural habitats, and in non-infected birds. However, the relationship was negative in urban habitats and in birds infected by *Haemoproteus*. Thus, it seems that birds under stressful conditions (i.e., infected or living in urban habitats) and with higher body condition (i.e., with access to optimal resources) likely increase GPx and SOD activities to reduce ROM and thus, decrease oxidative damage. Nevertheless, the causality of this effect could not be assessed, and further experimental studies could help to improve our understanding of the role of body condition in the capacity of urban-dwelling species to deal with stressors of anthropic origin and parasite infections while keeping the oxidative equilibrium.

CONCLUSION

It is difficult to isolate the effects of disease from those of food shortages, external stressors, or other environmental conditions that may favour disease transmission or modify the trade-off between birds' tolerance or resistance to infections. Nevertheless, these results may indicate that living in anthropized habitats and being infected by blood parasites may impose higher costs due to an exposure to multiple stressors and the limited availability of resources. Summarising the results from two works, we show that physiological analyses could be better understood if analysed together. Lastly, more experimental studies are needed to further comprehend the causality of results found in the field.

ACKNOWLEDGEMENTS

I am grateful to all the co-authors of the original publications Martina Ferraguti, Josué Martínez-de la Puente, Ramón C. Soriguer, Caroline Isaksson and Jordi Figuerola. Moreover, I wanted to thank José Antonio Gil-Delgado and Edgar Bernat-Ponce for the organization of the 7th WGUS meeting and the opportunity to write this chapter.

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Factors that determine the presence of avian malaria in House Sparrows in the east of the Iberian Peninsula

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ABSTRACT

The House Sparrow (*Passer domesticus*) is one of the most common birds in human-altered environments worldwide. Despite this, a significant population decline has been recorded in most regions where this species is native since the last decades of the previous century. The Valencian Community, in eastern Spain, is one of the areas where this decline was first reported in the country. The causes of this decline remain unclear and have been attributed to several factors, including agricultural intensification, land use changes, predation, pollution, and disease. Avian malaria, caused by parasites of the genus *Plasmodium*, is considered one of the possible contributing factors, particularly in urban and suburban areas. It has also been shown that population growth rate is inversely proportional to infection intensity. The aim of the present study was to assess the prevalence of haemoparasitic infections in several House Sparrow populations across different localities in eastern Spain (Valencian Community). Furthermore, we used Generalized Linear Mixed Models (GLMMs) to explore which biological and/or environmental factors best explained the prevalence of infection, including age, sex, Scaled Mass Index (SMI), and habitat type (urban vs. rural). The overall prevalence of haemoparasitic infection was 11.9%, and no significant explanatory variables were identified in the GLMM. Malaria prevalence was similar to that reported in other regions of Spain but considerably lower than the prevalence recorded in other European countries.

KEYWORDS

Avian malaria, decline, infection, mosquitoes, *Passer domesticus*, *Plasmodium relictum*

INTRODUCTION

The House Sparrow (*Passer domesticus*) has a wide distribution due to its association with humans, being common in towns, cities, and cultivated areas (Anderson, 2006; Summers-Smith, 1988). However, the species has been in decline since the mid-1970s within its native range. For example, in Great Britain, there has been a 60% reduction in population numbers, mainly in rural areas, although declines have also been observed in suburban and urban zones (Summers-Smith, 2003). In the city of London, the species is now practically extinct (Sanderson, 1999; Summers-Smith, 2005). In Spain, House Sparrow populations have declined by nearly 20% since 1998 (Murgui, 2022). An earlier study already documented a 90% decline between 1975 and 2001 in orange groves of eastern Spain (Gil-Delgado et al., 2002). Despite this, the species is not listed under any legal protection framework at either national or regional level in Spain. As in much of Europe, it has disappeared from areas where it was once abundant (Bernat-Ponce et al., 2018, 2020; Gil-Delgado et al., 2002; Murgui & Macias, 2010; Vera & Escandell, 2016).

The causes of this decline remain unclear, and several hypotheses have been proposed (Anderson, 2006). In rural areas, the decline appears to be more directly linked to changes in agricultural practices and land use (Hole et al., 2002). Agricultural intensification, including increased pesticide use and more efficient grain harvesting, reduces food availability and indirectly affects invertebrate populations, which are essential for feeding (Hole et al., 2002; Summers-Smith, 2003). These changes negatively affect survival rates, especially among first-year chicks (Murgui, 2022; Summers-Smith, 2003). In urban and suburban areas, a wider range of potential causes has been suggested, including predation, the loss of nesting sites in modern buildings, competition with other species for food, traffic, and environmental pollution, including noise pollution (Anderson, 2006; Bernat-Ponce et al., 2021, 2024; Chace & Walsh, 2006; Dadam et al., 2019; Murgui & Macias, 2010; Peach et al., 2018; Summers-Smith, 2003). Consequently, the decline in urban areas appears to be multifactorial rather than attributable to a single cause (Anderson, 2006). Diseases, particularly those caused by parasites, are also considered a possible contributing

factor. The House Sparrow is known to frequently suffer from diseases such as salmonellosis and mycoplasmosis (Anderson, 2006; Vincent, 2005). One increasingly prevalent parasitic infection is avian malaria, caused by hemoparasites of the genus *Plasmodium*, which have been shown to have demographic impacts on this species. A 2019 study in London found that 74% of sparrows were infected with *Plasmodium relictum*, the parasite responsible for avian malaria, and that the population growth rate was inversely proportional to infection intensity (Dadam et al., 2019).

The aim of the present study was to assess the prevalence of haemoparasites—particularly those responsible for avian malaria—in several House Sparrow populations across different localities in eastern Spain (Valencian Community). Furthermore, we investigated which biological and/or environmental factors best explained prevalence patterns. Our hypothesis is that the prevalence observed will be consistent with findings from other studies conducted in Spain and other European countries (Bichet et al., 2014; Dadam et al., 2019; Ferraguti et al., 2018).

MATERIAL AND METHODS

Study area

The study was conducted during 2020 and 2021 at seven sites across the Valencian Community (Fig. 1). Four of the sites were located in rural areas (Alcoy: 38°41'48"N 0°27'29"W; Sagunto: 39°42'24"N 0°14'21"W; Sueca: 39°14'33"N 0°18'54"W; Catarroja: 39°23'33"N 0°22'54"W), while the remaining three were situated in urban environments (Alcoy: 38°41'51"N 0°29'16"W; Valencia: 39°28'48"N 0°24'41"W; Meliana: 39°31'30"N 0°20'52"W).

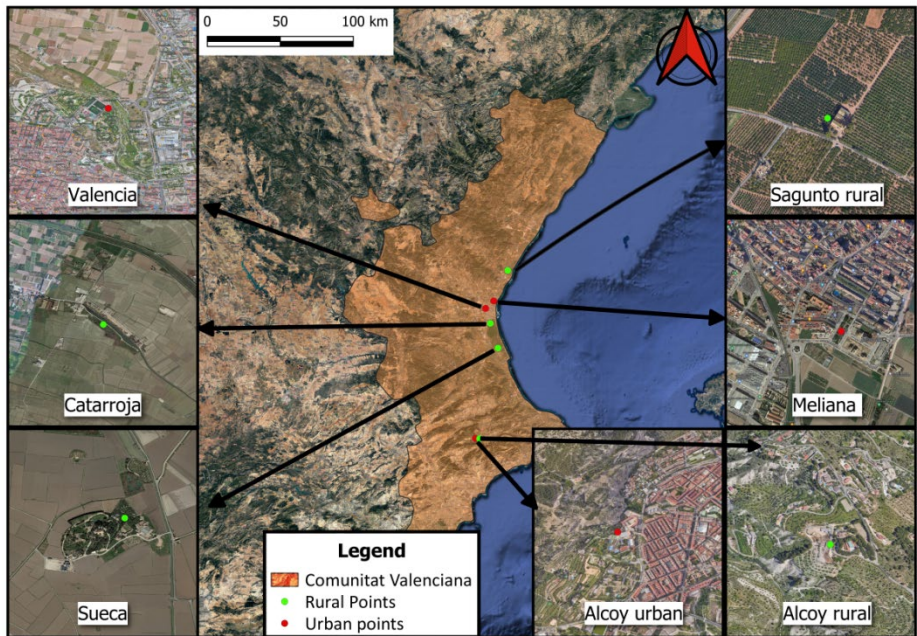


Figure 1. Map of House Sparrow (*Passer domesticus*) study sites in the Valencian Community (orange), indicating rural (green) and urban (red) sampling points.



Figure 2. Mist net with several House Sparrows captured in the rural area (Peluca Equestrian Center) of Alcoy.

Fieldwork and sample collection

House Sparrows were captured using nine-meter mist nets (Fig. 2). Each captured individual was banded with an official metal ring (SEO/BirdLife) to avoid repeated sampling. The following biometric data were recorded following Svensson (2009): body weight, sex, age, third primary feather length, wing length, fat index, and muscle index.

For blood collection, disposable 1 ml syringes pre-heparinized and fitted with 25 G needles were used. A 0.05 ml blood sample was drawn from the jugular vein of each individual. Sterile gauze was applied to the puncture site immediately after extraction to stop the bleeding, and the bird was released once bleeding had ceased. The specimens were released after ensuring that the bleeding had stopped.

The first drop of blood obtained was used to prepare a blood smear on a properly labeled microscope slide. Rounded-edge slides were used to avoid damaging red blood cells (Owen, 2011). Two blood smears were made per individual. Slides were then stored in appropriate containers for later fixation and staining in the laboratory.

Laboratory work

The samples obtained in the field were fixed within 24 hours by immersing them in 100% methanol for 3 minutes. Subsequently, they were stained with Giemsa at a 1:10 dilution (1 part Giemsa to 9 parts PBS) for 25 minutes, followed by rinsing with distilled water. Staining was completed within a maximum of one week after blood extraction (Dadam et al., 2019; Richard et al., 2002). Microscopic examination was conducted using an Olympus BH microscope at magnifications of $\times 200$, $\times 400$, and $\times 1,000$ for approximately 20 to 50 minutes per sample. At least 10,000 erythrocytes were observed at $\times 1,000$ magnification. If the hemoparasite *Plasmodium* spp. was detected, the sample was classified as positive for avian malaria.

Statistical analyses

To examine the sex ratio and assess potential sex bias in the samples, chi-square (χ^2) tests were performed for each locality. We also tested whether the sex distribution differed significantly across localities (Zar, 1984). To evaluate whether malaria prevalence was influenced by environmental or biological factors, we used a generalized linear mixed model (GLMM) with a binomial distribution. The dependent variable was the presence or absence of malaria infection. Fixed independent variables included: zone (rural or urban), age code (EURING CODE: 0 Unknown, 1 Male, 2 Female), sex (EURING CODE: 0 Unknown, 1 Male, 2 Female), and Scaled Mass Index (SMI) to account for size.

The SMI is a condition index formulated by Peig and Green (2009) that standardizes body mass relative to structural body size—in this case, using tarsus length. It adjusts each individual's mass to the expected value for a standard body size and is considered a reliable indicator of energy reserves, health status, and overall quality (Bernat-Ponce et al., 2023; Herrera-Dueñas, 2017; Peig & Green, 2009). Sampling site (i.e., locality) was included as a random effect. The statistical approach followed a previous study by Bichet et al. (2014). The “glmmTMB” function of the “glmmTMB” package (Brooks et al., 2017) was used to fit models. All the analyses were carried out in RStudio 4.1.0 (R Core Team, 2021).

RESULTS

Of the 165 House Sparrows captured (Table 1), 77 were males, 62 females, and 26 juveniles (sex undetermined). No significant differences were found in the proportions of males and females across localities ($\chi^2 = 7.98$, $p = 0.24$, $df = 6$). In none of the localities did the sex ratio deviate significantly from 1:1.

The overall haemoparasite infection rate among the captured House Sparrows was 11.9%. When analyzed by habitat, prevalence was 13.79% in rural areas and 10.26% in urban areas. However, the GLMM results indicated no significant differences in malaria prevalence with respect to sex, age, Scaled Mass Index (SMI), or habitat zone (Table 2).

Table 1. Summary of House Sparrows captured at the seven study sites in the Valencian Community, categorized by sex. The number of infected individuals in each category is shown in parentheses.

	Site						
	Alcoy Rural	Alcoy Urban	Catarroja	Meliana	Sagunto	Sueca	Valencia
Undetermined	0	15 (1)	0	2	4 (2)	1	4
Males	19 (2)	5	12 (2)	14 (4)	3	11 (1)	13 (1)
Females	12 (1)	6 (1)	5 (1)	12 (1)	9 (1)	11 (2)	7
Total	31 (3)	26 (2)	17 (3)	28 (5)	16 (3)	23 (3)	24 (1)

Table 2. Summary of the GLMM results evaluating factors affecting malaria prevalence in House Sparrow populations in the Valencian Community.

Ind. Variables	Estimate	z	p-value
Age (Young)	0.79	0.74	0.46
Sex (Male)	0.76	0.82	0.41
SMI	-17.84	-1.43	0.15
Zone (Urban)	-0.96	-1.37	0.17

DISCUSSION

The prevalence of the haemoparasites, did not differ between habitats (rural vs. urban) in the study areas of the eastern Spain. We also found no differences in prevalence associated with sex, age, or physical condition. In contrast, a study conducted suburban London reported a prevalence of 74% of *P. relictum* infection (Dadam et al., 2019). Similarly, research in France found prevalence rates ranging from 35% to 40% in urban and rural habitats, respectively (Bichet et al., 2014). In southwestern Spain, *P. relictum* prevalence was reported at 29.6% (Ferraguti et al., 2018).

Our results are more consistent with those from southwestern Iberia, with both regions showing prevalence rates approximately four to seven times lower than those observed in France and the UK (Bichet et al., 2014; Dadam et al., 2019). Furthermore,

Rivera et al., (2013) found no haemoparasite infections (*Trypanosoma* spp. and *Haemoproteus* spp.) in House Sparrows from the Albufera Natural Park, which includes two of our rural sampling sites (Catarroja and Sueca). These findings support the view that haemoparasite prevalence in House Sparrows is generally low in the Iberian Peninsula.

The similar prevalence rates across habitats in our study may be explained by comparable vector abundances—specifically *Culex pipiens* mosquitoes—across both urban and rural areas. One possible explanation for the overall low infection rates is the intensive agricultural activity surrounding all study sites. The widespread use of insecticides in these areas likely reduces mosquito vector populations (Summers-Smith, 2003). In fact, Culicidae mosquitoes are infrequently found in samples from the Albufera of Valencia (SEO/BirdLife, 2021). Vector control alone can substantially reduce haemoparasite transmission (Merilä et al., 1995; Sol et al., 2000). For example, both observational and experimental studies in pigeons have shown that vector abundance is a major factor influencing spatial variation in haemoparasite prevalence (Sol et al., 2000).

Our findings are also consistent with results from France, where prevalence was not predicted by any of the host traits investigated—such as age, sex, SMI, or wing length—and did not differ significantly between urban and rural habitats (Bichet et al., 2014). Some studies have suggested that males are more susceptible to parasitic infections due to reduced immune responses during the reproductive season, influenced by elevated testosterone and corticosterone levels (McCurdy et al., 1998; Rolff, 2002; Van Oers et al., 2010). In females, changes in immune function and exposure to vectors during incubation may also influence infection risk, though they might simultaneously experience increased mosquito bites during this period (Bichet et al., 2014; Jones et al., 2024). Age is another factor known to affect immune response. Young birds often suffer more infections than adults due to immature immune systems and prolonged exposure to vectors (Deviche et al., 2005; Huang et al., 2020; Hudson & Dobson, 1997; Sol et al., 2000). Similarly, an individual's body condition may influence susceptibility, as poor nutrition can promote parasite

proliferation and transmission (Beldomenico & Begon, 2010; Bichet et al., 2014; Palacios et al., 2012; Shurulinkov et al., 2012).

Although literature exists supporting the influence of sex, age, and condition on haemoparasite prevalence (Calero-Riestra & García, 2016; Jiménez-Peñuela et al., 2019; Jones et al., 2024; Lachish et al., 2011), our study did not detect any significant effects of these factors. A likely explanation is that the intense use of insecticides in agricultural areas reduces mosquito populations, limiting opportunities for infection and thereby masking any influence of individual host traits.

LIMITATIONS

We acknowledge that the present study presents a limited sample from a student's project and that a regular sampling in breeding and non-breeding season in each site would also be desirable in further studies to test avian malaria prevalence, given that *Plasmodium* parasitemia typically increases from February to September, peaking in autumn (Mora-Rubio et al., 2023). This research would also have improved using molecular methods, which would avoid much of the human error component in the detection/identification of *Plasmodium* spp. and *Haemoproteus* spp. (Richard et al., 2002).

CONCLUSIONS

House Sparrows exhibited a low prevalence of malaria in the studied areas, consistent with findings from other regions of Spain. None of the environmental or biological factors examined showed a significant effect on malaria prevalence. Further studies, conducted year-round and using molecular approaches, are needed.

ETHICS STATEMENTS

This study was conducted in accordance with all relevant institutional, regional, and national guidelines for the capture, ringing, and sampling of animals. Bird ringing was performed with permission from the Centro de Migración de Aves of SEO/BirdLife (Spain)

and the landowners of the capture sites. The capture and blood sampling of individuals were authorized by the Generalitat Valenciana—Conselleria d'Agricultura, Medi Ambient, Canvi Climàtic i Desenvolupament Rural (Authorization Reference Number: FAU20_007).

ACKNOWLEDGEMENTS

This study was a continuation of the Master's Final Project of C.dB.M. We are grateful to Carlos Nuévalos for his assistance during both field sampling and laboratory procedures.

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Challenges for occurrence and existence in urban ecosystem for House Sparrow

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ABSTRACT

House Sparrow (*Passer domesticus*) is a broadly distributed synanthropic species across the world. Over the past two decades, the population of the species declined in the urban ecosystem due to several ecological and anthropological reasons. In the study, we assessed the population status, habitat suitability, and genetic diversity of the species from selected states of the western part of India. We recorded 652 individuals (11.54 individuals/ha) across the three study sites of Gujarat i.e. Ahmedabad, Rajkot and Junagadh. Habitat suitability analysis using eBird.org records (2014-2022), the species appears to have a narrow niche breadth in summers than in winters, preferring majorly human settlement areas only. Niche dynamics indicate that summers are probably linked with the breeding time of house sparrows thereby showing contracted niches in comparison to the winters. The concatenated data of 1855 base pairs were used to measure the haplotype, nucleotide, and pairwise distance between the sampled populations. It was also observed that species were thriving in urban areas, particularly in areas with old buildings and open scrub areas; however, they were less common in areas with high-rise built-up structures. The questionnaire survey conducted to gather human perception on the importance of sparrows in the ecosystem/usefulness, population status and the causes of their decline highlights the need for open scrub patches, feeders, water baths, pesticide regulation, and regular population monitoring to maintain the House Sparrow population in urban and rural areas.

KEYWORDS

Abundance, Genetic Diversity, Habitat Suitability, Urban Ecosystem

INTRODUCTION

House Sparrows (*Passer domesticus*), belonging to the family Passeridae, are the most widely distributed species around the world especially in Eurasian and the Afrotropical realms (Mahesh and Lanka, 2021) with 28 species of Old-World sparrows (Päckert et al., 2021). The native range of the House Sparrow includes most parts of Europe, central Asia and North Africa (Hanson et al., 2020). As House Sparrows are human-commensal species and have been acquainted with human settlements since historic times (Summers-Smith, 1988; Vincent, 2005) hence, they were also introduced in other continents by humans (Schrey et al., 2011). Being globally common species and abundant in numbers, they are evaluated as “Least Concern” under the IUCN Red List (BirdLife International, 2019).

Human activity in the last few decades has led to unprecedented decline in biodiversity at an alarming rate ultimately leading to the loss of species and their abundance and the situation continues to be grim (Pyles et al., 2018). In India nearly 64.13% of the country’s population lives in rural areas (Trading Economics, 2024). The level of urbanization has increased from 27.81% in the 2001 Census to 31.16% in the 2011 Census, while the proportion of rural population declined from 72.19% to 68.84%. At this global change, it is very critical for a species to adapt to changes in its immediate environment. There is also evidence that climate change will contribute to species extinctions and will have a significant impact on the species distributions as well (Ikeda et al., 2017).

The similar impact and a drastic decline in the House Sparrow population has been reported from different parts of the world (Berigan et al., 2020; De Laet and Summers-Smith 2007; Erskine, 2006; Mahesh & Lanka, 2021; Mohring et al., 2021). Several hypotheses are put forth in order to explain the population decline of House Sparrows such as rampant urbanization (Modak 2017; Shaw et al., 2008), lack of nesting sites due to modern building designs and food supply (Pandian, 2018), air pollution, increased traffic, changes in its optimal habitat (Bernat-Ponce et al., 2020; Murgui & Macias, 2010),

indiscriminate use of pesticides (de Montaigne & Goulson, 2020; Dhananjayan et al., 2011), decrease in invertebrate food especially during breeding season (Peach et al., 2008), and predation by feral cats which also threatens sparrow population (Baker et al., 2005; Churcher & Lawton, 1987). Balmori & Hallberg (2007) reported, the electromagnetic radiation and the exhaust of unleaded petrol are also suspected causes of the disappearance of sparrows, however, no study clearly reveals how far electromagnetic radiation affects the behavior and breeding of sparrows (Mahesh & Lanka, 2021).

The conservation of House Sparrow is important because it has proved to be well suited for studies on environmental change, general biological problems including pests, and assessing key urban bioindicators (Hanson et al., 2020; Herrera-Dueñas et al., 2014). In the present study, we assessed the population status, habitat suitability, genetic diversity of House Sparrow (*Passer domesticus*) from selected landscapes in Northern and Western states of India (i.e. Gujarat, Rajasthan, Haryana and Uttar Pradesh). For the population status and genetic diversity study we could only sample select districts in Gujarat due to capture permit restrictions.

MATERIAL AND METHODS

Study area

Initially four states, i.e., Gujarat, Rajasthan, Haryana and Uttar Pradesh were selected for this study but finally it was conducted in three districts of Gujarat i.e. Ahmedabad, Rajkot and Junagadh (Fig. 1) only due to logistics and administrative reasons. However, the habitat suitability of the species has been mapped for all the above-mentioned states and for three seasons i.e., winters, summer and monsoon. In Gujarat, Ahmedabad, is the largest city and a seventh largest metropolitan area of India. Junagadh district is located on the Kathiawar peninsula in western Gujarat, whereas Rajkot is situated in the Saurashtra region of Gujarat and is the fourth largest city in Gujarat (Mehta, 2018).

The study sites have a hot semi-arid climate with hot, dry summers from mid-March to mid-June and the wet monsoon season from mid-June to October, and winters from November to February. Gujarat receives 670 mm of rain on average, although this rainfall varies greatly from year to year (Dewar & Wallis, 1999). The region experiences a lot of rainfall and high-speed winds during the monsoon season as well during months of May and June. During summertime, the temperature ranges between 24°C and 42°C and varies between 10 and 22 °C during winters (Lunagaria et al., 2015).

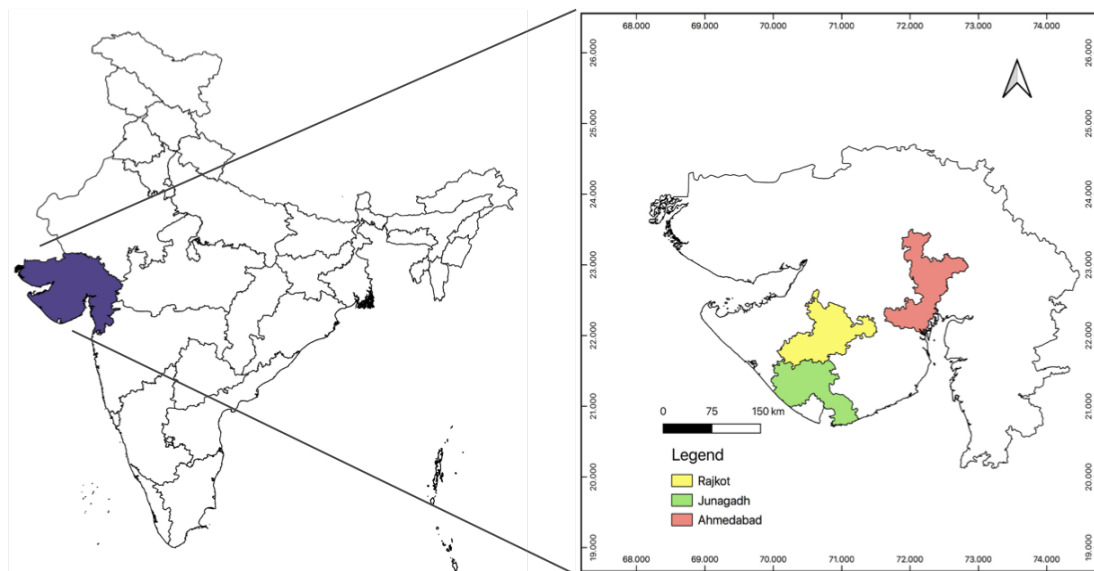


Figure 1. Map of Gujarat showing our intensive study sites, Ahmedabad, Rajkot and Junagadh for House Sparrow population and density estimation using point counts.

For estimating the House Sparrow population and density, Point Count methods (Bibby et al., 2000) were carried out in March 2022. We used fixed (50 m) radius point count to record the abundance of House sparrow for which we laid random points in urban and rural areas (Dhanya, 2011) throughout the district, using QGIS 3.10 mapping software (Fig. 2). These points were plotted taking the reference of potential points from eBird database following Bibby et al. (2000). A total of 72 points were sampled for House Sparrow abundance estimation, across the three districts for 18 days (Fig. 2). Each point was sampled for 10 minutes, within a 50 m radius. The minimum distance between two neighboring points was 200 m and the maximum distance was > 5 km (Mahesh & Lanka, 2021). All the point counts were sampled only once. The fieldwork was conducted in the morning, just after sunrise from 6:30 to 11:00 hours in the evening, when the sparrows usually return to their roosting sites just before sunset from 16:00 hours to 18:00, when the House sparrows were found to be most active (Patel & Dodia, 2021). Sampling was performed by a two-person team where the observer was the same during the entire sampling period. We recorded the sex, cluster size and habitat type at each sampled point count. A questionnaire survey was also conducted to gather information on the current status of House Sparrows, perceptions of their population decline, and views on the importance of sparrows in the ecosystem or their usefulness to humans. All age groups from 18 to 60 years were targeted.

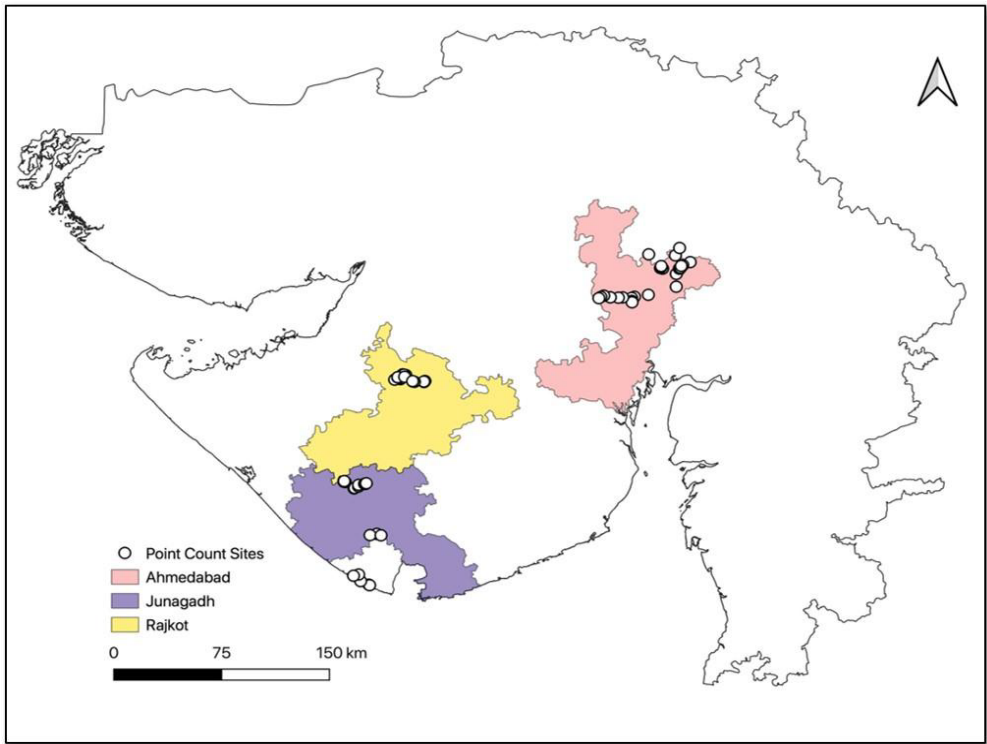


Figure 2. Sampling points in the study sites were Ahmedabad, Junagadh and Rajkot.

For assessing the habitat suitability of House Sparrows across the four states of India we applied presence-only ecological niche model techniques (Fig. 3). The occurrence data of house sparrow in these regions were obtained from eBird, India for 9 years (2014 to 2022) which were taken as presence points. eBird (Sullivan et al., 2009) is a citizen science platform that focuses solely on the avifaunal distribution (Sarkar et al., 2020). The occurrence data was directly downloaded from eBird (<https://ebird.org/data/download>). All overlapping and duplicate records were removed using ArcMap. We filtered all the records to obtain one occurrence record that was at least 1 km apart using the “spThin” package (Aiello-Lammens et al., 2015). This procedure reduces sampling bias and improves model performance. The WorldClim climatic variables were obtained at 30arc second resolution for further use as predictors in the model. These include monthly climatic variables derived from monthly precipitation and minimum and maximum temperature

that describes seasonality in climate that may be biologically important. The climatic data for each month was obtained from the WorldClim database (<https://www.worldclim.org/data/worldclim21.html>) and two other data sets; NDVI and Night time light were taken for modeling. All the data was pre-processed according to the needs of the model. Pearson correlation tests were conducted taking all the rasters of environmental variables before proceeding further with the habitat suitability model (Fig. 3). The data with correlation < 0.8 were carried forward for habitat suitability modeling. Maxent (Maximum Entropy Species Distribution Modelling) (Phillips et al., 2006) is one of the algorithms for presence only data in Species Distribution Modelling (Elith et al., 2006). This algorithm was chosen as the performance is comparable with other algorithms and requires only occurrence data (Elith et al., 2006; Kaky et al., 2020). ENMeval package was used in R (Muscarella et al., 2014) to estimate the distribution of House Sparrow in the selected study extent. To predict the environmental conditions occupied by species in proportion to their availability in the landscape, it requires a set of background points or pseudo-absence records where species occurrence was not recorded (Elith et al., 2011). A set of 10000 background points were randomly generated over the extent of House Sparrow predictions. For Maxent models (Phillips et al., 2006), the two main parameters to tune when calling ENMevaluate are (1) the range of regularization multiplier values and (2) the combinations of feature classes (Elith et al., 2011; Merow et al., 2013). ENMevaluate (3) builds different models for each combination of regularization multiplier (RM) values and feature class combinations.

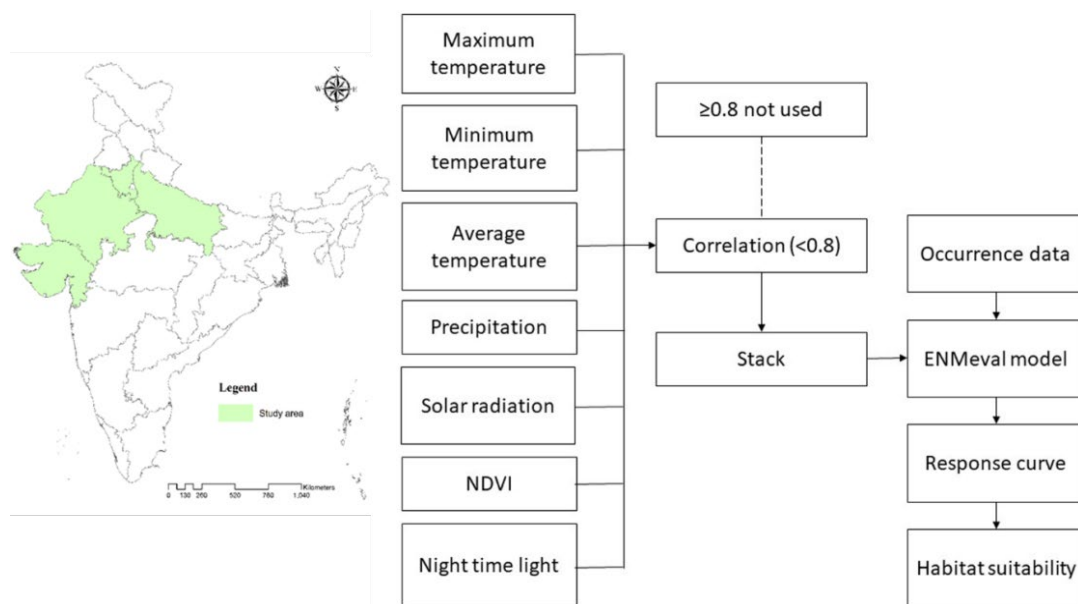


Figure 3. Methodological flow chart adopted to model the habitat suitability for House Sparrow.

The downloaded raster data are pre-processed to the same extent, so that there is no error occurring while running the model. We optimized MaxEnt parameters using the “ENMeval” package (Muscarella et al., 2014) to incorporate all combinations of 10 RMs (0.5–5.0 with 0.5 intervals) and three feature classes (L: linear, Q: quadratic, P: product), resulting in approximately 90 models.

Once the results were obtained, one or more models that are optimal across all the models which are run were selected. Some occurrence data may lie outside the study area extent which must be removed. Once that is completed, correlation check is done for the data, and the data < 0.8 correlation is considered for further modelling. The data with correlation < 0.8 is then stacked before running the ENMeval model. For model cross-validation, we used the “block” partitioning method that improves spatial and temporal transferability (Hijmans, 2012). As a result of the 'block' method, data is divided into four equal bins based on the latitude and longitude lines that divide the occurrence locations

and according to the position with respect to these lines, both occurrences and backgrounds are assigned to each of the four bins (Muscarella et al., 2014). For Maxent models (Phillips et al., 2006), the two main parameters to tune when calling ENM evaluate are (1) the range of regularization multiplier values and (2) the combinations of feature classes (Elith et al., 2011; Merow et al; 2013). ENM evaluate builds separate models for each unique combination of RM values and feature class combinations. Models were built using RM values ranging from 1 to 6 and with five different FC combinations (L, LQ, LQH, LQPT, LQP; where L = linear, Q = quadratic, H = hinge, P = product and T = threshold). The model's complexity is analysed under different parameter combinations, to select a combination with lower complexity for modelling (Zhao et al., 2021). The optimal model is selected considering validation tests using the lowest average AICc value with the highest average validation AUC. Model performance was evaluated using the area under the receiver-operating curve (AUC), which is generally considered good at 0.75 and very good at 0.90 (Rodriguez-Ruiz et al., 2018).

Further, to study genetic diversification, mist-netting was carried out to collect House Sparrow blood samples. Upon capture, a drop of blood was collected on FTA card by pricking the branchial vein of the bird with a sterile needle (Gutiérrez-Corchero et al., 2002; Hoysak and Weatherhead, 1991). In total, fifteen individuals were captured, five each from Nanodara village (Ahmedabad), Dandhusar village (Junagadh) and Vajdi village (Rajkot), using mist nets to obtain blood samples (1-2 µl). After collecting the blood sample, the individuals were released in their native habitat. Sampling sites for mist netting were chosen based on the movement pattern of the bird.

DNA was isolated from blood on FTA cards using DNeasy® Blood & Tissue Kit (Qiagen, Inc.) protocol. DNA isolates were stored at -20°C. Amplification of DNA sequence was performed for three mitochondrial genes i.e., 16S, Cyt *b* and ND2. Reaction volume for PCR was taken upto 10 µl containing 1 µl of BSA, 0.2 µl of each primer, 0.2 µl of taq, 1 µl of taq buffer, 0.2 µl of dNTPs, 6.2 µl of nuclease free water and 1 µl of DNA template. The primer sequence and PCR conditions for the three genes are followed as provided in Sorenson et al. (1999).

Amplified DNA sequence was visualized into 2 % agarose gel under Gel doc system (Griffith, 1998).

PCR amplified amplicons were cleaned using ExoSAP-IT™ and cycled sequenced with Big Dye™ terminator version 3.1 (Applied Biosystem, Foster City, CA) using Applied Biosystem Genetic Analyser 3500 (Applied Biosystem Foster City, CA). We amplified the sequence for 16S, Cyt *b* and ND2. Sequence was generated by ABI 3500 that were checked using Finch TV for quality and editing in case of mismatch. For data analysis sequence alignment and gap filling was carried out using MEGA 11 (Tamura et al., 2021). Muscle alignment for all the sample sequence for each gene was carried out in MEGA 11 software (Tamura et al., 2021). Samples with all three genes were chosen to merge the sequence in order to get large DNA sequence data. Haplotype and nucleotide diversity, and pairwise distance were analyzed in DnaSP 6 software (Rozas et al., 2017). The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1855 positions in the final dataset. Evolutionary analyses were conducted in MEGA 11 (Tamura et al., 2021).

RESULTS

During the survey, out of a total of 72 points selected all along three districts (Table 1), House Sparrows were sighted only at 44 points. A total of 652 individuals were encountered. In Ahmedabad, we counted a total of 249 individuals, with the highest number of individuals in the rural region, especially at Point number 25 at Nalsarovar circle, where we counted 36 individuals (Fig. 4). Similar patterns were observed in Junagadh, where the highest number of individuals was seen in the rural region, especially at Point number 10 at Dhandhusar village where we counted 32 individuals. In Rajkot, the highest number of individuals was seen in the semi-urban region, especially at Point number 03, where we counted 33 individuals. The number of males and females were also counted to assess the male-female sex ratio; the same is represented in Fig. 5. Surprisingly, sparrows were observed to be thriving even in the urban areas in Ahmedabad (Fig. 6). Sparrows were seen in most of the areas of Old city, Ahmedabad, especially in Gheekanta region. No sparrows were observed in Junagadh city limits. Further moving towards Girnar Wildlife Sanctuary, where the region is dominated with dry deciduous scrub forests, no sparrows were detected. Most of the sparrows were seen in Sasan-Gir village. Male and female sex ratio in Junagadh and Rajkot appeared to be almost even, which could possibly indicate the breeding pairs and the onset of breeding season.

Table 1. The number of sampled bird point counts (50 m radius) surveyed in each district with respective density of House Sparrow

Place	Total Points	Male	Female	Total Individuals	Individuals/ha
Ahmedabad	31	88	161	249	10.23
Junagadh	21	76	85	161	9.77
Rajkot	20	135	107	242	15.41
Over All	72	299	353	652	11.54

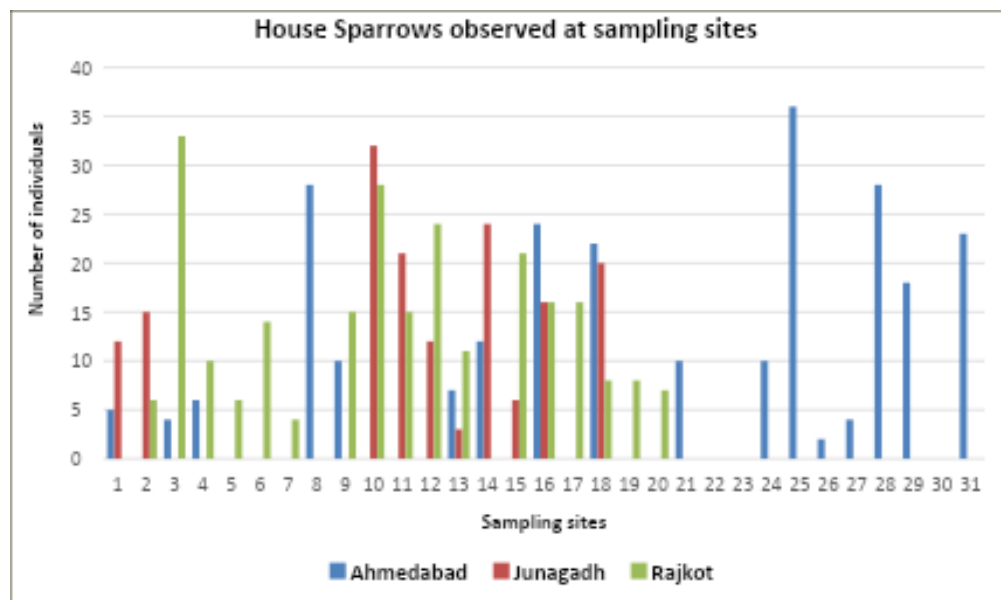


Figure 4. Number of House Sparrow individuals observed during point counts at the sampling sites in Ahemdabad, Junagadh and Rajkot districts of Gujarat.

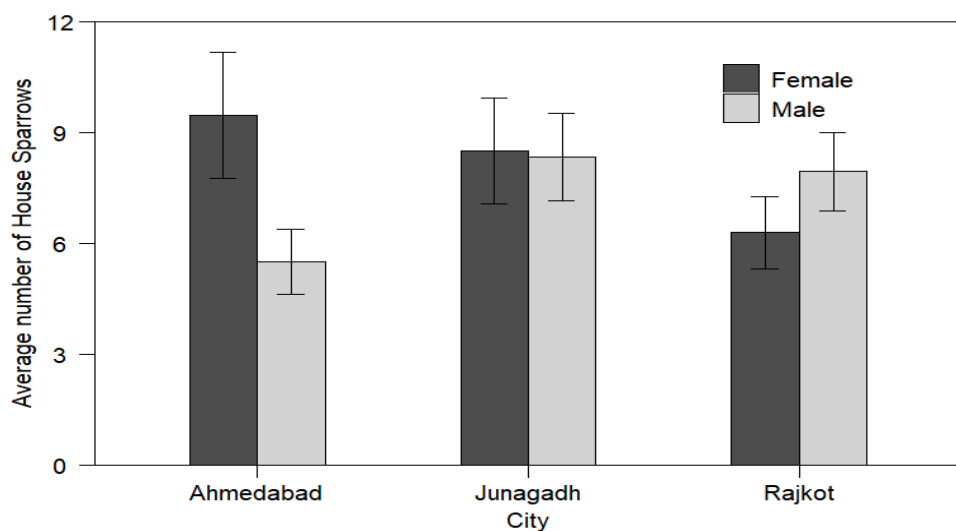


Figure 5. Male:Female sex ratio of House Sparrows observed at the study sites.



Figure 6. (a) House Sparrows observed feeding along with Common Babblers and Common Mynas in Ahmedabad; (b) Male and Female House Sparrow observed near the nest box in a hotel, Rajkot district; (c) House Sparrow feeding on a sieve (spicy salty mixture) at a Tea shop, Junagadh district.

Questionnaire results indicate the presence of House Sparrows in the past in Ahmedabad (Table 2), whereas declining alarmingly especially from few parts of the core regions of the city. Interviewers opined that House Sparrows have disappeared since the advent of cell-phone towers and digitalization, construction of modern buildings and urbanization.

Table 2. Opportunistic interviews conducted on the cause of decline of House Sparrow in Ahmedabad.

Age Group	Number	Comment
18-30	7	Decrease in sparrow population due to Electro-magnetic radiations from cell towers and urbanization.
40-50	11	Decrease in sparrow population since the advent of cell-phone towers and reduction in nesting spaces. Sparrows used to visit houses, ten to twenty years back.
60-70	5	Presently they have disappeared due to the use of mobile phones. Major decrease in population since ten years.

The E-Bird data was used to model the distribution of House Sparrow to predict its habitat suitability in Gujarat, Rajasthan, Haryana and Uttar Pradesh for winter (December, January, February), summer (March, April, May) and monsoon, (June, July, August) for nine years (2014-2022). The House sparrow winter habitat suitability for nine years (Fig. 7), showed spatially dynamic shifts in areas where in 2014 and 2015 medium suitability areas covered large area of extent bordering Gujarat and Rajasthan and Kutch. The high suitability areas were typically surrounding all highly urbanized clusters in all the states. Medium and high suitability areas increased in Gujarat and Rajasthan over the years. The year 2019 and 2020 showed higher habitat suitability near urban clusters than medium suitability areas. The increasing urbanization trend in arid and semi-arid states of Gujarat and Rajasthan could be one of the reasons for showing such patterns of habitat suitability in House Sparrows. Minimum temperature and maximum temperature had correlation > 0.8 and hence it was not taken into consideration for the model. FC combination of LQH with RM values of 1, 2 and 3 was the best model as per the AUC value. LQH with RM value of 1 was the best model for seven of the nine years. In summer (Fig. 8), majority of the area comes under low and high suitability is only limited to the settlement regions. Average temperature for summer had high correlation (>0.8) in all years and was excluded in the model. The best model obtained was the LQH and LQPT with RM values of 1, 2 and 3, having LQH with RM value 1, the most common one. When it comes to the monsoon season (Fig. 9), the suitability in each year is different. Model which was best suited for monsoon was LQH and LQPT with RM values 1, 2 and 3. Same as summer suitability, average temperature was excluded from the model as it had correlation > 0.8 . When comparing all the data, the FC combination of LQH with RM value 1 was the most common in all the habitat suitability models. Upon observing the habitat suitability map of House sparrow across all years, it appears that most of the high suitability areas are seen in and around the settlement areas. The habitat suitability maps in summer are significantly different from other seasons showing few areas with high and medium habitat suitability in 2014 and expanded in terms of area coverage in 2015 in Gujarat and in the following years

towards Uttar Pradesh. Suitable areas significantly reduced in 2020 and then increased in Gujarat and Uttar Pradesh. In monsoon (Fig. 9), the House Sparrow habitat suitability patterns were significantly different from other seasons. It was observed that the spatial spread in high suitability areas was comparable with medium suitability areas in monsoon. This was observed in Gujarat and Rajasthan in 2014, and then gradually increased towards Rajasthan and Haryana in 2016. Habitat suitability drastically decreased in 2017 and then concentrated in Haryana in 2021 and spatially both medium and high suitability areas were the highest in 2022. The high dynamism in the spatiotemporal niche in House sparrow certainly indicates many clustered areas how high suitability in monsoon whereas medium suitability areas are significantly less in summer than in winter. In monsoon it is observed that the urban, semi-urban and rural zones are highly suitable while in summer, suitability is clustered more in urbanized centres than in winter.

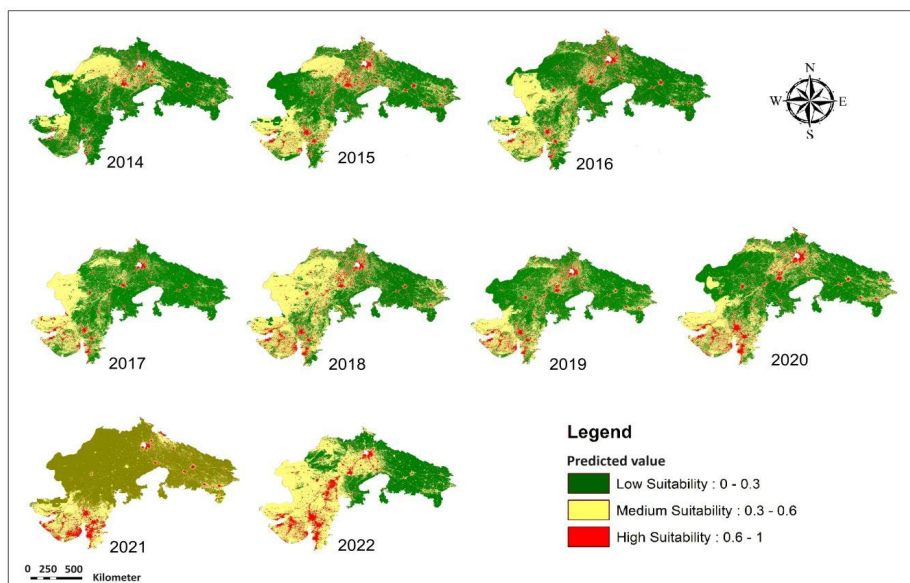


Figure 7. Winter (December, January, February) House Sparrow habitat suitability maps of four states (Gujarat, Haryana, Rajasthan and Uttar Pradesh) for the years 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, and 2022.

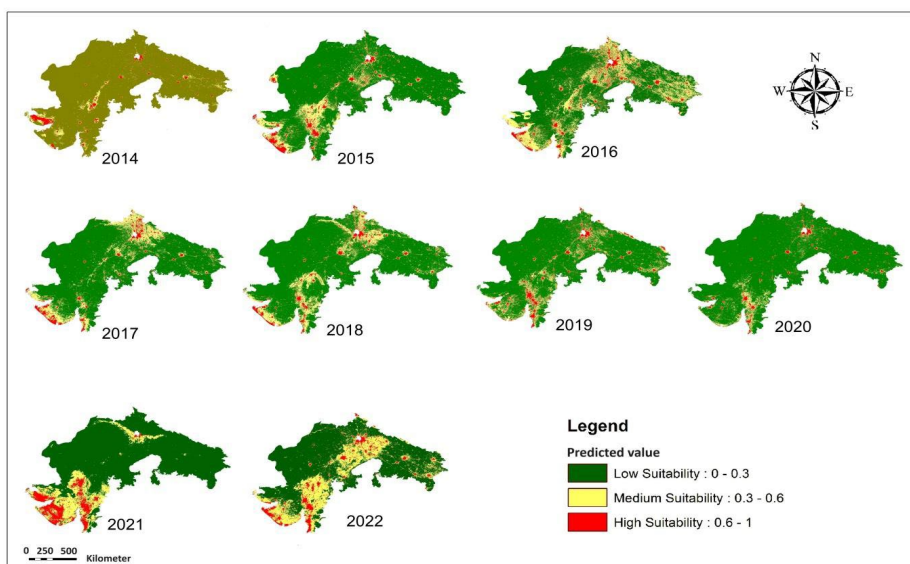


Figure 8. Summer (March, April, May) House Sparrow habitat suitability maps of four states (Gujarat, Haryana, Rajasthan and Uttar Pradesh) for the years 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, and 2022.

The concatenated data of the generalised sequence yielded 1855 base pairs long sequence of *P. domesticus* mitogenome. The phylogenetic tree of the samples shown in Fig. 10, indicates that the individuals from Junagadh and Rajkot form one clade whereas Ahmedabad formed another. This could be an artifact of sampling as Junagadh and Rajkot are comparatively closer than the Rajkot and Ahmedabad. Adding more samples covering the entire landscape would result in more accurate results and changing phylogeny. Table 3 shows the results of gene flow and genetic diversity of house sparrow obtained from mitochondrial genes. Number of haplotypes in Ahmedabad was 5 (n=5), Junagadh 7 (n=7) and Rajkot 4 (n=4). Nucleotide diversity for Ahmedabad, Junagadh and Rajkot was 0.084, 0.054, and 0.128 respectively (Tables 3, 4 & 5).

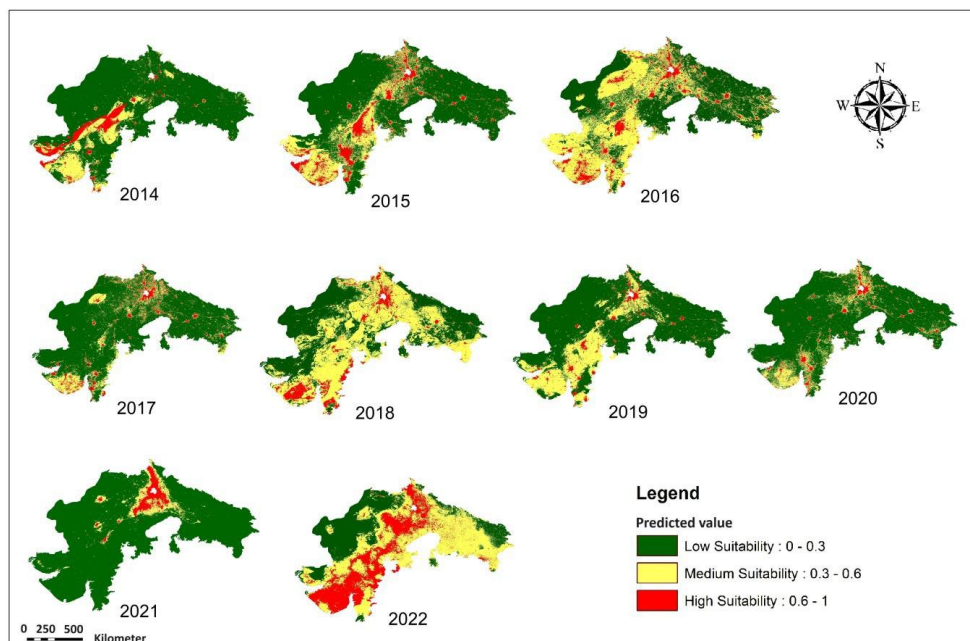


Figure 9. Monsoon (June, July, August) House Sparrow habitat suitability maps of four states (Gujarat, Haryana, Rajasthan and Uttar Pradesh) for the years 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, and 2022.

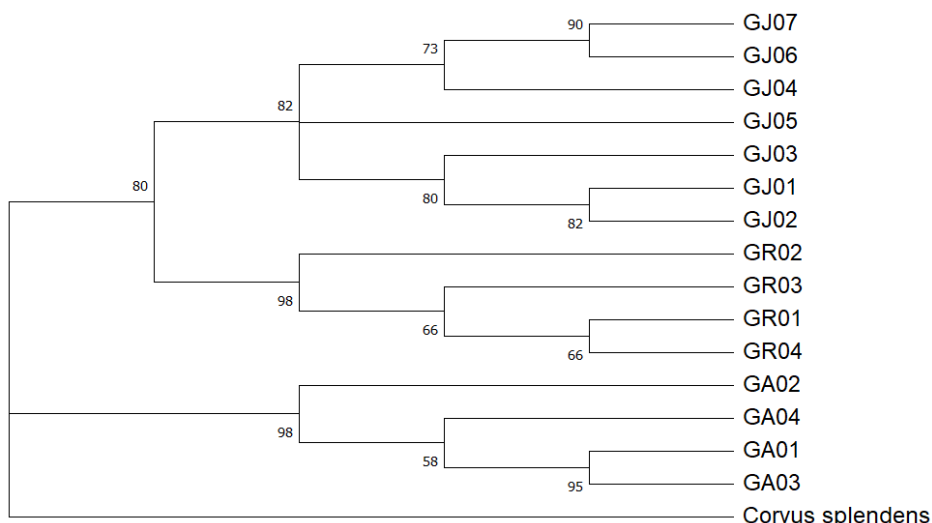


Figure 10. The Neighbour Joining Phylogenetic tree showing the relationship of the populations (Ahmedabad (GA), Rajkot (GR) and Junagadh (GJ)) in Gujarat using MEGA 11 software.

From the pairwise distance analysis and the obtained sequences we have seen individuals from Junagadh population ($P=0.04$) having greater gene flow as compared to the individuals from Ahmedabad population ($P=0.09$). Further, the genetic diversity is higher in Junagadh population as compared to Ahmedabad.

Table 3. Haplotype diversity for 16S rRNA, Cyt *b* and ND2 gene). For Rajkot Cyt *b* gene data is not shown as the sample size was less than 03. **n**: number of samples; **Hn**: number of haplotypes; **Hd**: haplotype diversity; **π** : nucleotide diversity.

Gene	Location	n	Hn	Hd	π
16S rRNA	Ahmedabad	5	5	1.0	0.087
	Junagadh	7	7	1.0	0.054
	Rajkot	4	4	1.0	0.128
Cyt <i>b</i>	Ahmedabad	3	3	1.0	0.190
	Junagadh	7	7	1.0	0.134
	Ahmedabad	4	4	1.0	0.031
ND2	Junagadh	7	7	1.0	0.019
	Rajkot	4	4	1.0	0.107

DISCUSSION

In the current study, a total of 652 individuals were sighted, and the House sparrow sightings were comparatively higher in rural regions than urban areas. The occurrence of the species in rural areas could be attributed to the availability of human-made structures, suitable for nesting such as roof cavities, crevices walls and nest boxes (Maxmellion & Priyatharsini, 2021). Individuals were also sighted near small hotels, eateries and provisional shops as they offer plenty of anthropogenic food items. In semi-urban areas, individuals were observed in open lands with scrub vegetation and garbage dumping sites adjacent to residential spaces. These green spaces could be providing nesting sites and insect food for the young (Summers-Smith, 1988). House Sparrows living near residential areas consume more human refuse and household scraps (Summers-Smith, 1988). During the survey, nesting sites were observed to be located at small tea shops, eateries and hotels where there are abundant food sources. Surprisingly, sparrows were observed to be thriving even in a few urban areas in Ahmedabad. Sparrows were seen in most of the areas of the city, Ahmedabad, especially in Gheekanta region. Interview results indicate the presence of House Sparrows in the past, whereas some are declining alarmingly, especially in few parts of the city. As Junagadh is mainly comprised of dry deciduous scrub forests, with a forest area of about 1345.1 Km² the forested regions showed minimum sparrow presence. Most of the Sparrows were seen in Dhandhusar and Sasan-Gir village, in higher numbers. The male-female breeding pairs were seen together in most of the points especially near the nest boxes.

Few study sites in metropolitan settings include vegetable markets and food grain stores because sparrows typically favour these locations as a good source of spillover food materials. However, the lack of habitat in these locations may be the main cause of the sparrow population decline in urban areas. According to the reports, House Sparrow populations have been plummeting over the past few years, mostly in large cities (Patel and Dodia, 2021). The State of India's Birds Report (2020) also made note of the fall in House Sparrow populations in urban environments. The primary causes of population decline are

reported to be shortage of nesting sites brought on by widespread urbanisation (Bokotey and Gorban 2005) and lack of thrashed food resources from domestic households (Moudrá et al., 2018). Several additional variables, such as electromagnetic radiation and the exhaust from vehicles using unleaded fuel, could possibly be contributing to the decline of House sparrows (Balmori and Halberg, 2007; Summers-Smith, 2007). The rationale for their population fall is still up for debate, though. The richness of House Sparrow populations is mostly attributed to the availability of a variety of food sources for adults and nestlings as well as crucial nesting locations close to the food sources. Rajashekhar and Venkatesha (2008), recommend ideal nest locations for House Sparrows through the preservation of native and ancient buildings in the city, open areas, artificial nest boxes, and reducing pesticide use in urban areas. Rural and semi-urban areas have less development pressure than urban areas, but they nonetheless provide ample space for urban sprawl. As a result, these places are vulnerable to further development pressure in the future, and sparrows in rural areas may lose their habitat quite easily. The GIS data across all years indicate most of the high suitability areas in and around settlement areas. It appears that House sparrow has a narrow niche breadth in summer than in winter. Such, niche dynamics explain that summer months are probably linked with the breeding time of house sparrows thereby showing contracted niches in comparison to the winter season, as also reported by Bernat-Ponce et al. (2018) in urban areas of southeast Spain. To prevent local extinction of the species and to develop effective conservation policies, we must regularly monitor the species to compare and investigate the population fluctuations along the gradient (Narayanappa et al., 2022). In the current study we were only able to sample three districts of Gujarat and estimated genetic information at the local-scale; however, the genetic study at the broader landscape-scale will help us understand the genetic diversity, gene flow and the landscape level resistivity for the preparation of species-specific management and conservation action plans.

ACKNOWLEDGEMENTS

We greatly acknowledge the funding support from the Ministry of Environment, Forest and Climate Change, Government of India through the Grant-in-Aid support. We also thank the Principal Chief Conservator of Forest and Chief Wildlife Warden for granting permission to conduct the work. We thank the Divisional Forest Officers of Ahmedabad, Rajkot and Junagadh for their suggestions during the work. We also thank Edgar Bernat-Ponce for providing valuable comments on the article. We extend our sincere thanks to the residents of Ahmedabad, Rajkot and Junagadh for responding to the questionnaire survey.

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Tree Sparrow nests as indicators of plastic pollution in anthropized environments

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ABSTRACT

Nowadays, plastic stands out as one of the most widespread pollutants in natural environments. While much research has focused on plastic pollution in marine ecosystems, it is essential to recognize that plastic also affects terrestrial ecology. Birds, which construct nests using a variety of locally available materials, are not exempt from this influence, as they sometimes incorporate plastics of human origin into their nesting materials. This study aims to investigate whether the tendency to include such anthropogenic materials also extends to birds that build nests in enclosed spaces, such as nest boxes. To explore this, we examined a total of 11 Tree Sparrow nests, categorizing the materials used and paying particular attention to those derived from human activities, especially plastics. In addition, we analyzed whether the birds exhibited preferences for specific colors and types of anthropogenic materials when constructing their nests, which could be influenced by the availability of these materials in the local environment. Our findings revealed that plant-based materials were the primary components of the nests, followed by animal-derived and anthropogenic materials. Notably, anthropogenic materials were present in all the nests examined. Interestingly, there appeared to be a distinct preference for black and filamentous anthropogenic materials over other types and colors. This suggests that tree sparrows may be selective in their use of human-made materials—possibly due to factors such as local availability, physical properties like durability, or resemblance to naturally occurring materials.

KEYWORDS

Anthropogenic material, bird nest, filamentous, plastic, tree sparrow.

INTRODUCTION

Plastic is one of the most prevalent pollutants generated by human activity in the natural environment (MacLeod et al., 2021). It can be found across a wide range of ecosystems, with marine environments providing most of the studies (Blettler et al., 2018; Monteiro et al., 2018; Reddy et al., 2018; Thushari & Senevirathna, 2020; Bergmann et al., 2022). Therefore, the use of plastic materials in nest construction has been widely observed in seabirds (Jagiello et al., 2019; 2023; Montevecchi, 1991; Provencher et al., 2015).

Although there are fewer studies, the use of plastic (or anthropogenic material in general) in nest construction is also known in terrestrial birds (Batisteli et al., 2019; Jagiello et al., 2019; Luna et al., 2022; Mallet et al., 2020; Møller, 2017; Ryan, 2020; Wang et al., 2009). Scarce research delves deeply into the plastic found in nests, often merely detecting the presence or absence of certain items without examining the possible preferential selection by birds (Briggs et al., 2023). The amount and type of anthropogenic material that birds use in their nests can vary depending on the degree of urbanization of the environment (Janic et al., 2023). In urban areas, the most common types of man-made material usually are cigarette butts and fragments of plastic bags (Carbó-Ramírez et al., 2015; Jagiello et al., 2022, 2023; Potvin et al., 2021; Reynolds et al., 2016; Suárez-Rodríguez et al., 2013; Wang et al., 2009). In agricultural environments, plastic strings are commonly used for specific tasks, so they are often available in the surrounding area (Jagiello et al., 2018). Nest collection in these environments has indicated that a significant portion of them contains plastic, so it is considered one of the most common anthropogenic materials used as a nestling material in these areas (Avery-Gomm et al., 2012; Batisteli et al., 2019; Biddle et al., 2017; Broughton & Parry, 2019; Jagiello et al., 2018; Norman et al., 1995; Sergio et al., 2011; Townsend & Barker, 2014; Zdunaik et al., 2021).

Because of this, the prevalence of anthropogenic material in nests can be used as an indicator of pollution in the environment (Jagiello et al., 2023; Tavares et al., 2016; Zduniak et al., 2021). However, their utility will depend on the nest-building bird's ability to selectively choose or preferably avoid certain contaminants due to its type, shape, size, or

colour, among others (Batisteli et al., 2019). In cases where a preferential selection of certain types of human-made material for nest construction has been detected (Jagiello et al., 2018; Suárez-Rodríguez et al., 2013; Votier et al., 2011) hypotheses have been formulated regarding the potential functions it may serve. Outstanding among the non-structural functions are intraspecific signalling of builder quality (Jagiello et al., 2023; Sergio et al., 2011), protecting offspring against parasites (Mainwaring et al., 2014), or serving as an olfactory component (Bonadonna & Mardon, 2013; Jagiello et al., 2023).

The objective of this study is to investigate if nests from nests-boxes could also serve as indicators of plastic pollution in anthropogenic environments. We aimed to study the availability of environmental plastic to compare it with the plastic debris found in the nests and determine whether there is selection or avoidance of plastic depending on their shape and colour. To accomplish this, we collected nests built in nest boxes used by the Tree Sparrow (*Passer montanus*), which are widely distributed in the eastern region of the Iberian Peninsula (Gil-Delgado et al., 2005; Ponz et al., 1996).

MATERIAL AND METHODS

Study species

The Tree Sparrow (*Passer montanus* Linnaeus, 1758) is a small bird (not exceeding 14 cm) belonging to the family Passeridae. It does not exhibit visible sexual dimorphism. It is a troglodyte bird as it takes advantage of any natural or human-made cavity to build its nest. That's why it readily accepts the installation of nest boxes. The shape of the nest is ovoid, the base is usually made of grass, twigs, and stems, and the interior is lined with feathers, hair, wool, and often artificial materials like plastics and strings (García-Navas, 2016).

Study area and nest collection

A total of 11 Tree Sparrow nests were collected, when abandoned, from nest boxes located in an orange grove area of Sagunto, Valencia, province in east of Spain nearby of shoreline of Mediterranean Sea at the end of the 2023 breeding season. These

orange cultivations have been repeatedly described in other studies (Alambiaga et al., 2020; Barba & Gil-Delgado, 1990). Since the 1980s, nest boxes have been consistently provided each year for species that use cavities for breeding, primarily the great tit (*Parus major*), although they are also used by other species, including mammals (Barba & Gil-Delgado 1990; Barba et al., 1995; Gil-Delgado et al., 2006).

Each collected nest was naturally dried for at least one month at the Cavanilles Institute of Biodiversity and Evolutionary Biology (ICBiBE) terrestrial vertebrates' laboratory (University of Valencia). Each nest was weighed using a precision balance (± 0.1) to obtain the initial weight of the entire nest. Subsequently, nests were disassembled by tweezers in a laboratory tray following Espinoza et al. (2024). The materials comprising the nest were separated and categorized by type, with each category being re-weighed to obtain the partial weights. The materials from each nest were classified by type into grass straws, feathers, hairs, twig fibers, wool, moss, green leaves, decomposed leaves, conglomerate, and anthropogenic materials (Table 1). Each of these components was stored in zip-lock labelled bags with the nest's identifying code. All these materials were weighed (g), and based on the results, relative percentages were calculated to determine their representation for the total nest weight. To simplify data analysis, the initial 10 categories were regrouped into 4 main categories: vegetal material (grass straws, twig fibers, wool, moss, green leaves, and decomposed leaves), animal material (feathers and hair), anthropogenic material, and conglomerate (Table 1). Materials of anthropogenic origin underwent a more detailed analysis.

Each piece of anthropogenic material found was classified according to type (plastic, rope, or fabric), shape (filamentous, laminar, hard or foamy), and colour (black, transparent/white, red, green, blue, orange or brown). The prevalence of fabric, rope, and plastic in each nest was studied. Finally, the use of anthropogenic materials was estimated based on their shape and colour in relation to their availability in the study plots. To do this, the material found in the plots (data extracted from the work of Espinoza et al. (2024) carried out in the same study area) was compared to the material used that appeared in

the nests. This comparison was conducted based on both the shape (filamentous, laminar, hard, or foamy) and the colour (black, green, white, blue, orange, red, and brown). According to Espinoza et al. (2024), environmental availability were 50 plastic-based fragments in 26 (72.2%) out of 36 m² soil samples, with a variation of 0-8 pieces per sample (mean = 1.4; sd = 1.6; N = 36). For 1 ha in the orange groves of Sagunto, the number of plastic fragments potentially available for nest construction was estimated in 13,889 pieces (range = 11,209 - 16,569). Among the 50 pieces, 10 were filaments (20%) and 40 (80%) were non-filaments (46% hard fragments, 32% sheet; 2% foam). The most prevalent type of plastic in the environment were hard fragments. Six colours were identified in the orange groves samples, and white pieces were the most prevalent.

Category	Description
VEGETAL	
Grass straws	Herbaceous fragments, such as Poaceae or branch fragments.
Twig fibers	Filaments derived from the sheaths of the stems of herbaceous plants.
Wool	Woolly material primarily composed of tufts and similar plant-derived structures.
Moss	Small fragments of moss.
Green leaves	Dry leaves from plants, still displaying a greenish coloration.
Decomposed leaves	Dry leaves from plants in an advanced state of decomposition.
ANIMAL	
Feathers	Bird feathers and down.
Hair	Animal hairs.
ANTHROPOGENIC	
Anthropogenic material	Artificial materials such as plastic or strings.
CONGLOMERATE	
Conglomerate	Aggregated material consisting of nest remnants, primarily soil, very small herbaceous fragments, stones, bird excrements, and other debris.

Table 1. Description of each of the selected categories for the classification of materials found in the nests under study.

Statistical analysis

All the analyses were carried out using R and RStudio (R Core Team, 2021). To compare the weights of the four established categories (vegetal material, animal material, conglomerate, and anthropogenic material) and determine which category was predominant, means were calculated from data collected from different nests. After analyzing the normality (with “stats” package and “shapiro.test” function in RStudio) and homoscedasticity (with “car” package and “leveneTest” function) of these categories, a Kruskal-Wallis’ test was conducted (with “stat” package and “Kruskal.test” function). Subsequently, the categories were ranked using a post-hoc analysis, the Mann-Whitney test (with “stats” package and “wilcox.test” function) (Zar, 1984).

Prevalence values of plastic type and color of nests (N=11) and the environmental samples (N=36) were used to calculate the Manly’s Index (Manly et al., 2002). It computes the relationship between available and used values. So, it was used to evaluate the selection of plastic colours and types in nests. This was calculated using the function “wiRatioEJ” from the package “adehabitat HS” (Calenge, 2006).

RESULTS

After classifying all the separated material, we obtained a total of 10 initial categories (Table 2). When grouped into the four established categories, all nests contained human-made materials, indicating a 100% prevalence (Fig. 1).

Weight data is normal for “Vegetal Material” ($W = 0.983$; $p = 0.980$), “Animal Material” ($W = 0.958$; $p = 0.749$) and “Conglomerate” ($W = 0.947$; $p = 0.601$) categories but not for “Anthropogenic Material” ($W = 0.690$; $p < 0.001$). The homoscedasticity of this data cannot be significantly rejected ($F: 5.235$; $p = 0.004$). Based on the results of the Kruskal-Wallis test, the weight mean of at least one category is significantly different ($df = 3$; Kruskal-Wallis chi-square = 36.754 ; $p < 0.001$). The post-hoc analysis using the Mann-Whitney test determines that, except for the means “Conglomerate” and “Vegetal Material” ($p = 0.1$), the weight means are significantly different ($p < 0.001$). Means of the

“Vegetal Material” (49.9%) and “Conglomerate” (41.4%) group are higher than those of the “Animal Material” group (8.0%), and in turn, these are higher than those of the “Anthropogenic Material” group (0.8%).

Table 2. Summary of material of each of the 11 Tree Sparrow nests from nest boxes in orange groves of Sagunto (Eastern Spain). It shows the nest identification code (Nest ID); partial weight of each of the categories obtained for each nest, represented in percentages except for the initial weight, which the weight in grams is obtained before the decomposition of the nests; and the percentage of each category.

Nest ID	Initial weight (g)	Vegetal material						Animal material		Conglomerate	Anthropogenic material
		Grass straws	Twig fibers	Wool	Moss	Green leaves	Decomposed leaves	Feathers	Hairs		
E33	81.8	13.7	3.1	2.9	0.0	4.1	6.6	1.0	0.3	67.7	0.6
E427	93.7	11.2	13.3	4.3	0.0	2.1	6.8	11.2	0.0	50.9	0.1
C45	33.0	21.6	4.0	6.5	0.0	8.6	23.5	4.3	0.0	31.2	0.3
E458	47.3	19.3	3.3	0.0	0.0	23.1	0.0	3.5	0.2	50.3	0.2
N413	39.6	45.1	3.4	3.1	0.0	6.0	10.0	12.1	0.0	19.7	0.5
E497	54.4	16.6	3.7	7.8	0.0	4.9	10.4	8.6	0.0	47.0	1.0
M348	38.1	33.9	3.3	6.0	0.0	3.0	6.3	6.3	0.0	38.3	3.0
464E	40.0	21.2	8.7	1.9	0.0	9.2	7.3	11.1	0.3	39.7	0.5
C417	40.8	21.7	11.3	1.0	0.0	1.0	10.0	17.4	0.0	36.6	1.0
E360	37.1	35.6	1.4	0.6	0.0	7.9	14.1	2.5	0.3	37.3	0.3
276E	47.1	40.3	3.6	2.4	0.4	3.3	4.5	8.5	0.2	36.1	0.7

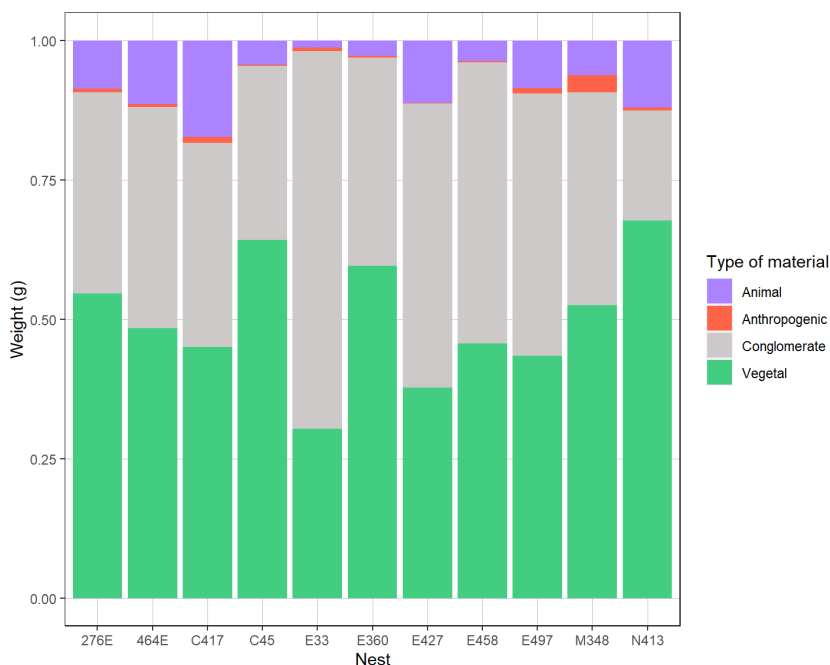


Figure 1. Relative proportion of the different categories that make up the analysed nests. It is noteworthy that the prevalence of anthropogenic material is 100%.

The presence of anthropogenic material in all nests is noteworthy. On one hand, regarding the type of anthropogenic material, plastic prevalence in nests is 100%, while the rope and fabric prevalence are 36.36% and 9.09% respectively. On the other hand, regarding the shape of anthropogenic material, filamentous prevalence in nests is 90.91%, while the laminar and foamy prevalence are 45.45% and 9.09% respectively.

Although its high presence, weight of anthropogenic material never exceeds 5% of the total nest weight (Fig. 1) and on average it was the 0.8% of the nest material. Within that 0.8%, 95.17% is plastic, 4.58% is rope, and 0.25% is fabric. Within the 95.17% of plastic, 98.40% is filamentous, 1.34% is laminar, and 0.27% is foamy. Therefore, within anthropogenic material, plastic is the primary contaminant in all nests, especially filamentous plastic (Fig. 2).

Manly's Index does not yield any significant p -value, either for the shape nor for the colour of plastic. In the case of plastic shape, the p -value is marginally significant for filamentous, and the W_i statistic is greater than 1 (p -value = 0.060; W_i : 2.674). For 'Hard' type and 'Orange' and 'Red' colours of plastic, the analysis does not generate any numerical value due to the absence of use in the nests (Table 3). However, 'Hard' type was clearly avoided by the species (Fig. 3) due to a great availability but no use to build the nests.

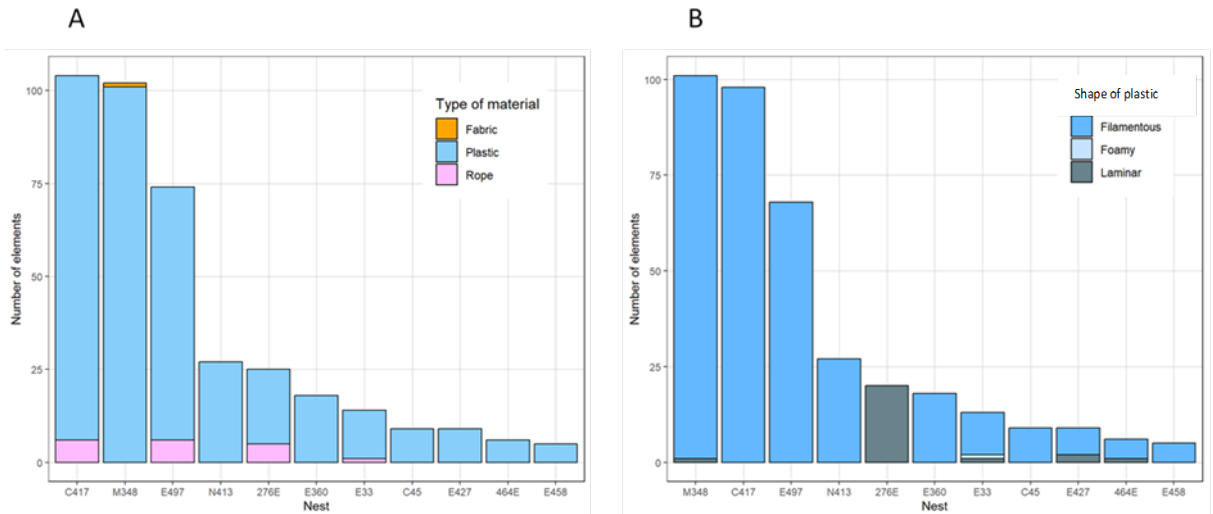


Figure 2. Absolute percentages of each type of anthropogenic materials (A) and each plastic shape (B) present in each nest.

Table 3. Statistics from the Manly's Index conducted to determine the use of different types and colours of plastic based on their availability in the study area. The 'Used' column shows the proportion of nests that contain each category of plastic type and colour, while the 'Available' column denotes the proportion of plots than contain those same categories. 'Wi' is the Manly selectivity measure (selection ratio: used/available) and SE.Wi is the standard error of the selection ratios. The *p*-value is denoted by an asterisk (*) if $p < 0.1$. Finally, 'Bi' equals $Wi/\sum(Wi)$.

Shape of plastic						
	Used	Available	Wi	SE.Wi	<i>p</i>	Bi
Filamentous	0.688	0.257	2.674	0.891	0.060*	0.478
Laminar	0.250	0.343	0.729	0.359	0.450	0.130
Hard	0.000	0.371	0.000	-	-	0.000
Foamy	0.062	0.029	2.188	3.022	0.694	0.391
Colour of plastic						
	Used	Available	Wi	SE.Wi	<i>p</i>	Bi
Black	0.417	0.293	1.424	0.488	0.385	0.353
Green	0.125	0.171	0.732	0.469	0.568	0.181
White	0.375	0.366	1.025	0.343	0.942	0.254
Blue	0.083	0.098	0.854	0.706	0.836	0.212
Orange	0.000	0.024	0.000	-	-	0.000
Red	0.000	0.049	0.000	-	-	0.000

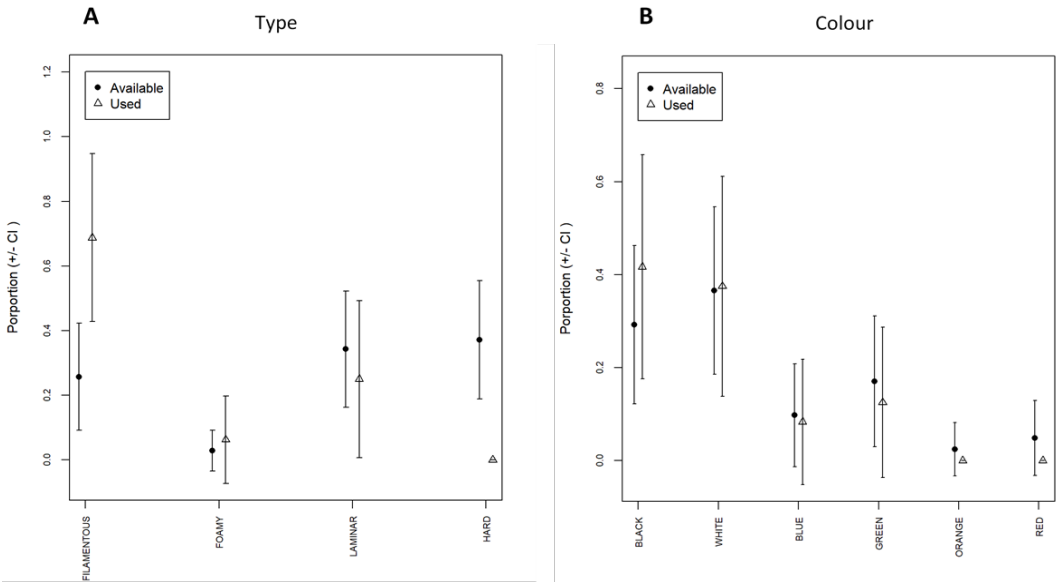


Figure 3. Used and available proportions of (A) types of plastic material (filamentous, foamy, laminar and hard), and (B) colour of plastic material (black, white, blue, green, orange and red).

DISCUSSION

The present study shows that anthropogenic material is present in all of the 11 nests, however, the predominant mass within nests is attributable to vegetative materials and conglomerate, particularly with a notable presence of grass strands. The results indicate that plastic is the main contaminant in these nests, especially filamentous ones. Despite this, we do not find a clear preference for any plastic type and colour. However, it seems that filamentous debris are selected and hard fragments avoided for nest construction.

Vegetal material comprises the bulk of the nest mass in our work. Quantitative data of the proportion of each material that make up the nest is relatively rare in the literature; however, grass and stems are among the most common (Deeming, 2023). According to Mainwaring et al. (2014), nests exhibit a categorizable composition comprising

two distinct types of materials, structural and lining material. Due to its shape, vegetal material could function as the first type, shaping and supporting the nest, maintaining the integrity of the structure (Deeming, 2018). Animal-origin elements such as feathers or hair, which are less abundant in our study, would act as lining materials, creating a microclimate suitable for offspring development, and providing insulation (Deeming, 2023; Hansell, 2000, 2005; Hilton et al., 2004).

Despite not being as abundant as other materials, anthropogenic-origin material stands out for being found in all the nests from the agricultural study area. The proportion of nests containing plastic has been found to be a useful indicator of plastic pollution and the prevalence of this material has been increasing in the last years (Jagiello et al., 2019, 2023; Ryan, 2020). A diverse range of avian species use plastic and other human-origin materials for nest construction (Jagiello et al., 2019; Ryan, 2020). While research has predominantly focused on seabirds, such as the brown noddy (*Anous stolidus*), terrestrial birds like the caracara (*Caracara plancus*), black kite (*Milvus migrans*), magpie (*Pica pica*) and the chinese bulbul (*Pycnonotus sinensis*) also incorporate this material into their nests (Jagiello et al., 2019; Mallet et al., 2020; Møller, 2017; Ryan, 2020; Wang et al., 2009).

In other study, carried out on Darwin's finches, plastic prevalence also reached 100% (Harvey et al., 2021), in accordance to our study. Our found plastic prevalence rates are higher than the ones obtained by Batisteli et al. (2019) in an orange orchard like our study area, but in Brasil. This difference could be since the study by Batisteli et al. (2019) was conducted with several species at once; however, ours only focuses on the Tree Sparrow. However, the mere presence of anthropogenic material in the nests does not necessarily imply that they can be used as pollution indicators in the environment. Sometimes birds can be selective regarding the type of material and even its colour, which could skew pollution estimates obtained solely from these elements (Batisteli et al., 2019).

Among all the man-made materials present in nests, plastic twine is one of the most problematic due to the risk of strangulation (Antczak et al., 2010; Seacor et al., 2014; Townsend & Barker, 2014). In our case, filamentous plastic is the main contaminant in the

nests. Nests come from nests boxes located in agricultural areas so, the appearance of this material could be because, like the availability hypothesis argue, it is present and accessible in the environment (Reynolds et al., 2019; Wang et al., 2009). Our results do not indicate a clear preference for any form of plastic, maybe due to the small sample size, since studies posit that a sizable sample is requisite for robust incidence estimation (Ryan, 2020). However, it seems to be a trend to choose filamentous material among others. That could lead us to think that maybe they may be confusing due to the resemblance of the plastic material to natural elements like grass or animal hair (dark and filamentous) (Antczak et al., 2010; Jagiello et al., 2023; Potvin et al., 2021; Ryan, 2020). Additional viewpoints could focus on the alterations in the natural environment, which could lead to reduce the availability of natural nestling materials, forcing individuals to search for substitutes (Jagiello et al., 2019). But there are some bird species, like the pied flycatcher (*Ficedula hypoleuca*) or some tits, that incorporate plastic twine into their nests even when they are provided with natural materials (Briggs et al., 2023; Broughton & Parry, 2019; Surgey et al., 2012). This acquisition could be beneficial (or at least not as harmful as believed) for the birds (Batisteli et al., 2019; Deeming, 2023). It seems that these anthropogenic elements might be easier to find and manipulate, reducing the nest-building time (Mallet et al., 2020; Ryan, 2020). Another explanation, not mutually exclusive, could be its utilization as a reinforcing material for the nest structure. Jagiello et al. (2019) concluded in their study that debris can be used for this purpose, so it is expected that filamentous elements may be employed to mitigate damage caused by extreme weather conditions such as heavy rain and strong winds (Antczak et al., 2010; García-Navas, 2016; Heenan & Seymour, 2011). We should consider that nests in our study come from nest boxes, so it is worth considering, apart from the mentioned small-sized sample, that there is not as much preference for selecting filamentous materials to reinforce the nest, as the nest would already be protected by the box. In some species, as the Eurasian Blue Tit (*Cyanistes caeruleus*), it has been demonstrated that the use of plastic (as well as wool and cotton) reduces flea levels, suggesting that this anthropogenic material could act as an antiparasitic element (Potvin et al., 2021; Reynolds et al., 2016).

In our study area, we found hard fragments; however, we did not detect their presence in any nest. Although the analyses may not be significant, it may make sense for them to avoid their use, as according to the literature, plastic is sometimes used as a communicative signal for conspecifics (Sergio et al., 2011). The fact that the nest is built inside a nest box, where minimal visual signals are expected, could be related to the avoidance of hard plastic and the apparent absence of a clear preference for the colour of the plastic used, as we see in our results. Some works about nests from outside of nestling boxes propose the 'signalling hypothesis' explaining that the selection of the colour of anthropogenic materials could be important in sexual selection in some birds. For example, the black kite includes white plastic that serves as a reliable marker of its viability, territory quality and dominance (Jagiello et al., 2023; Sergio et al., 2011). Plastic could attract conspecifics (Mallet et al., 2020; Sergio et al., 2011), but its use may entail a cost, as nests containing plastic may be more prone to predation (Møller, 2017). To verify if colour could indeed play an important role in these contexts, it would be advisable to compare our results with nests of the same species that do not come from nest boxes.

To summarize, plastic was found in all the analysed nests, but we cannot conclude that birds that built them had a preference for selecting any colour. However, they could have a trend to incorporate filamentous plastics, maybe because its similitude with natural materials and/or due to its alleged structural properties. Likewise, Tree Sparrows seem to avoid hard plastic fragments, perhaps it is because they build their nests inside nest boxes, so the visual properties of hard plastics would not be as useful as in other situations. In any case, it would be advisable that in future studies, with a larger sample size, attention be focused not only on verifying the presence of anthropogenic material in the nests but also on detecting a possible preference for the material and attempting to determine the causes of this preference.

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The House Sparrow (*Passer domesticus*) and the Great Tit (*Passer major*) as urban bioindicators in Belgium

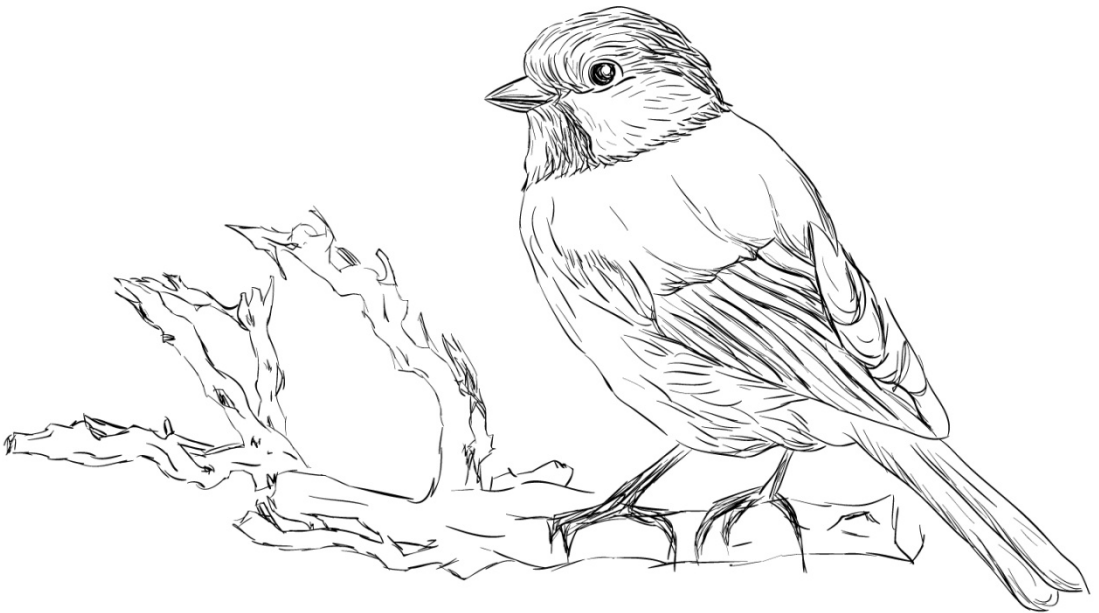
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The House Sparrow (*Passer domesticus*) and the Great Tit (*Passer major*) as urban bioindicators in Belgium

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ABSTRACT

In 2022, the size of House Sparrow (*Passer domesticus*) groups in Flanders (Belgium) remained small. More than 75% of all groups contained fewer than 10 breeding pairs. In many towns, fewer than 25% of the groups had more than 10 breeding pairs. Great Tits (*Passer major*) in cities began breeding earlier than their counterparts in forests. They laid one more egg on average, but their breeding success and chick condition were lower. Since urban breeding pairs often did not return in the following season, breeding in cities appears to be stressful. Despite the value of long-term monitoring programs like this one, 82% of counts remain one-off snapshots, which are less useful for tracking detailed trends over time.

KEYWORDS

Breeding success, Great Tit, House Sparrow, monitoring programs, urban ecology

INTRODUCTION

The proportion of the world's population living in cities is expected to exceed 66% by 2060 (United Nations, 2014), and in recent decades, many cities and even small villages have experienced significant urbanisation (Gorissen & Meynaerts, 2018). As a result, individual trees, small forests, green walls, hedges, and scrubland are steadily disappearing. This is not only a major issue for urban and global biodiversity, but it also creates several problems for people, including urban heat islands, smog alerts in summer, and increasing distances for city dwellers to reach the countryside. Consequently, there is a growing need to design cities in ways that benefit both biodiversity and human quality of life.

House Sparrows (*Passer domesticus*) have long been among the most common birds in Europe (De Laet & Summers-Smith, 2007; Summers-Smith, 1988). They were primarily found around farms in agricultural landscapes and in densely built urban areas. This social species forms loose, discrete breeding colonies, typically consisting of 10–20 pairs. It is also extremely sedentary by nature, rarely moving more than 100 m from the nest during the breeding season. Outside the breeding season, movements of up to 600 m are common, and young birds may occasionally undertake long-distance dispersal flights.

The Great Tit (*Passer major*) is also a territorial resident during the breeding season. Outside of this period, it often joins mixed-species flocks with other tit species (De Laet, 2008). The species readily accepts nest boxes, and in areas where these are abundant, Great Tits nest almost exclusively in them.

In 2002, a "National House Sparrow Day" was initiated through collaboration between ABLLO vzw, the research lab TERC (Ghent University), and Vogelbescherming Vlaanderen (VBV). This long-term study revealed a steady decrease in the average group size of House Sparrows in Belgium (De Coster, et al. 2015). In 2004, together with several international scientists, we established the International House Sparrow Group (WGUS), which has met regularly since 2007 (2007: London, UK; 2009 and 2011: Newcastle, UK; 2014: Ghent, Belgium; 2017: České Budějovice, Czech Republic; 2019: Breda, Netherlands;

and 2023: Valencia, Spain). During this period, we published an article (De Laet & Summers-Smith, 2017) and several meeting reports in the journal *International Studies on Sparrows* (De Laet et al., 2006, 2012; De Laet & Summers-Smith, 2017; Havlick & De Laet, 2016).

Finally, in 2014, we launched a research program on urban Great Tits in Flanders, covering one large metropolis (Ghent), two smaller provincial towns (Sint-Niklaas and Dendermonde), and one village center (Kruibeke). During this period, forest-nesting Great Tits were also monitored in a 30-hectare, 100-year-old beech forest equipped with 193 nest boxes since 1964 (Hutsepot, Zwijnaarde), which served as a control area for the study.

MATERIAL AND METHODS

House Sparrow study

Since 2002, VBV has annually launched a widespread call to count the number of chirping male House Sparrows during a single day at the end of March or the beginning of April. This provides a reliable estimate of the number of breeding pairs (De Coster et al., 2015). Participants are asked to classify the number of chirping males observed into one of seven categories (Table 1). The mean of the range within each category is used in subsequent statistical analyses. Additionally, participants are requested to provide supplementary information about the location—typically a garden—where the sparrows are counted.

Table 1. Summary of the categories used to classify the number of chirping male House Sparrows.

Category	Number of chirping House Sparrow males
0	0
1	1 – 5
2	6 – 10
3	11 – 15
4	16 – 20
5	21 – 30
6	>30

Great Tit study

Each participant in this study is provided with a standardised nest box, typically placed in private gardens, courtyards, or on apartment terraces. A limited number of nest boxes were also installed in cemeteries and school grounds. Starting in March, each nest box is checked weekly. The date of the first egg, the number of eggs and nests, and the number of fledglings per nest are recorded in a standardised format. Young birds are captured, ringed, and measured at approximately 15 days of age. Wherever possible, adult birds are also captured when the chicks are between 10 and 15 days old.

RESULTS

House Sparrows showed a steady decline in group size between 2002 and 2011. After 2011, group sizes appeared to stabilize, fluctuating between 5 and 10 breeding pairs per group (Fig. 1). When comparing group sizes above and below 10 pairs, we found that the proportion of small groups (<10 pairs) increased (from 77% to 80%), while the proportion of large groups (>10 pairs) decreased (from 23% to 20%) (Fig. 2).

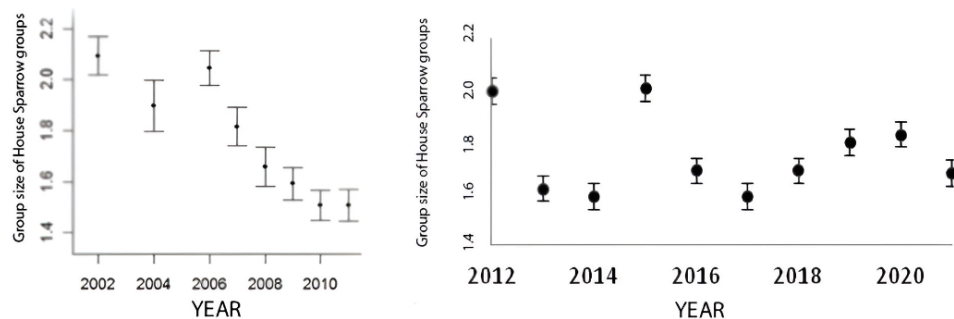


Figure 1. Left: Evolution of House Sparrows group size 2002 – 2011. Right: Evolution of group size 2012 – 2021.

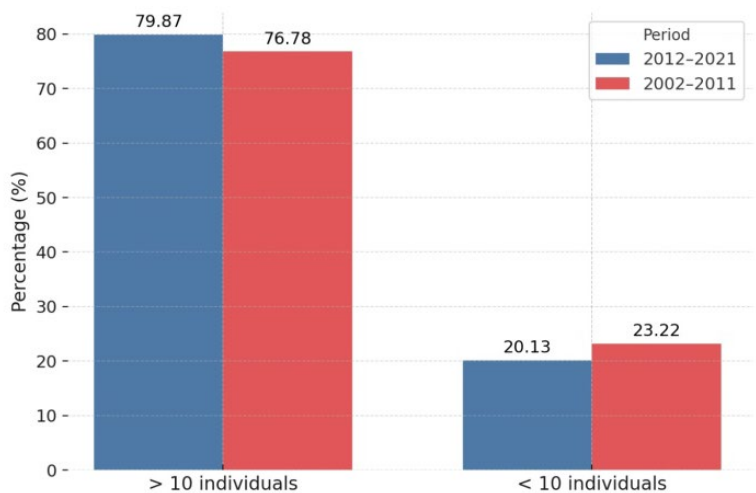


Figure 2. Percentage of House Sparrow group sizes > and < than 10 individuals between 2002 and 2021 in Belgium.

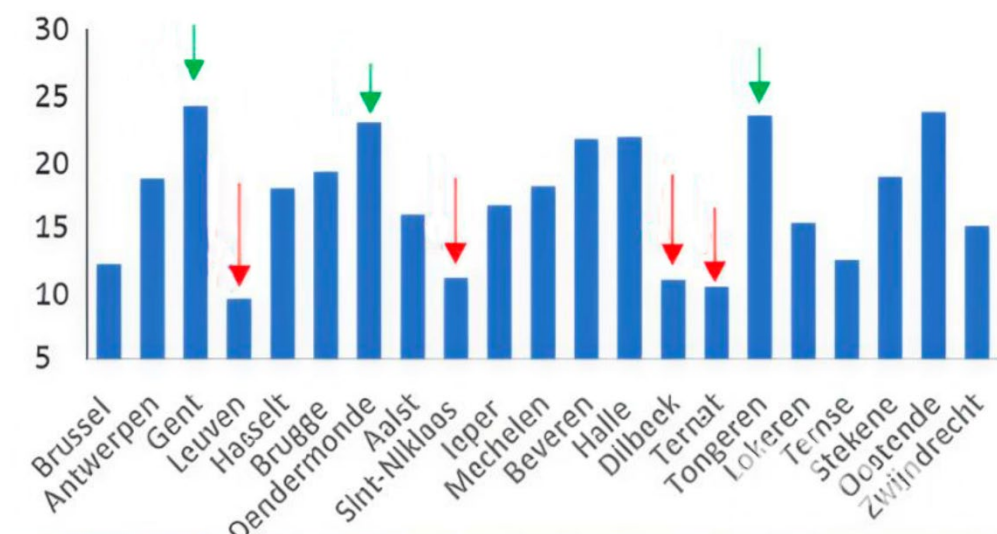


Figure 3. Percentage House Sparrow groups >10 in Belgium capital cities, small cities, and villages. Some cities did well (Gent, Dendermonde, Tongeren and Oostende), marked with a green arrow, and some cities were bad (Leuven, Sint-Niklaas, Dilbeek and Ternat), marked with a red arrow.

None of the surveyed towns had more than 25% of House Sparrow groups with over 10 breeding pairs (Figure 3), even though such group sizes are crucial for the long-term survival of colonies (Vogelbescherming Nederland, 2008). More than 50% of our data consists of small groups. Large groups, with more than 15 pairs, account for barely 10% of the total (Figure 4). One-off counts (82%) offer only snapshots and are less useful for tracking detailed trends over time. In contrast, repeated counts (18%) at the same sites are ideal for monitoring long-term changes.

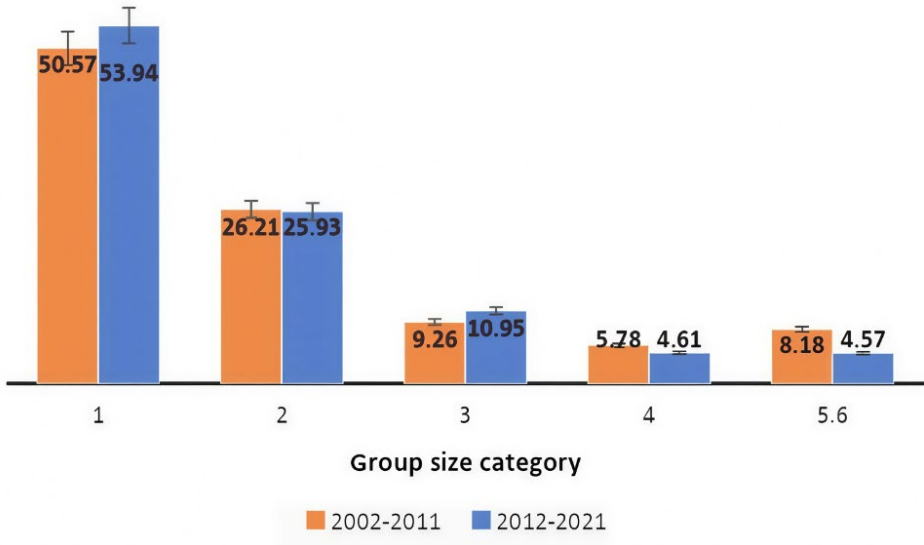


Figure 4. Percentage of group size category of House Sparrows between 2002 -2011 versus 2012 -2021.

Urban Great Tits began breeding significantly earlier than those in the control forest area (Fig. 5). This pattern was not observed in breeding Blue Tits. Notably, urban Great Tit females laid one more egg than their forest counterparts (Fig. 6). Although they laid one more egg, they had lower breeding success or higher nest mortality than forest-breeding great tits (Fig. 7).

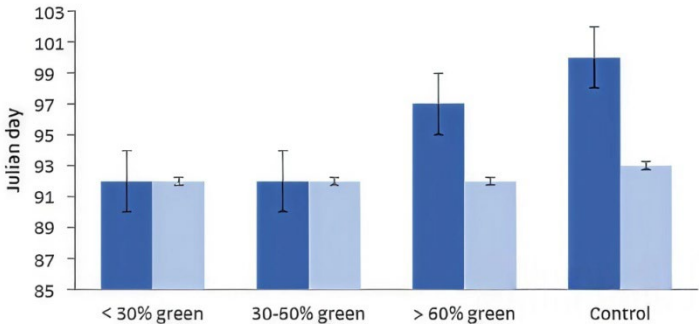


Figure 5. First egg dates (Julian day) of Great Tits and Blue Tits in different urban areas in Belgium. Control = forest area. *Parus major* (dark blue), *Cyanistes caeruleus* (light blue).

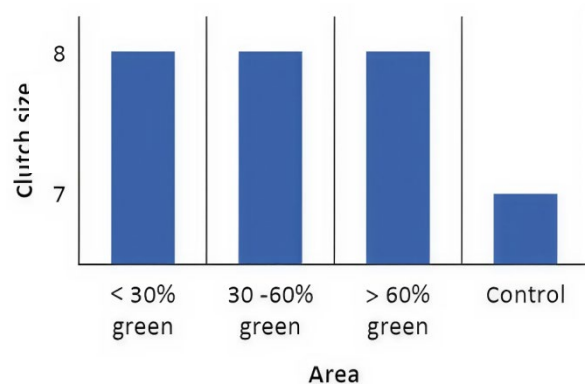


Figure 6. Clutch size of Great Tit females in different urban areas and the forest area (control).

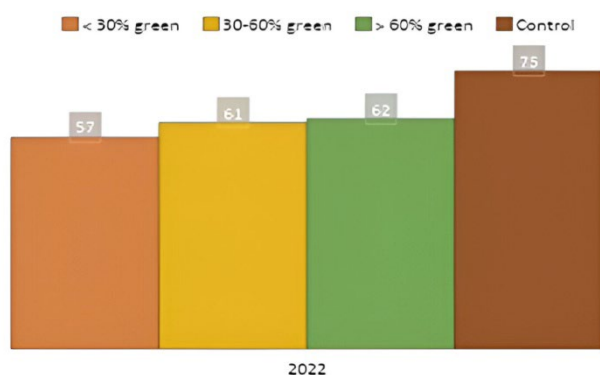


Figure 7. Breeding success (number of chicks) of Great Tits in different urban areas versus the forest control area in 2022.

We also assessed the condition of urban Great Tits at 15 days of age and found it to be poorer than that of forest-breeding individuals (Fig. 8). Furthermore, most urban Great Tit parents were young birds that did not return to breed the following year (personal observation).

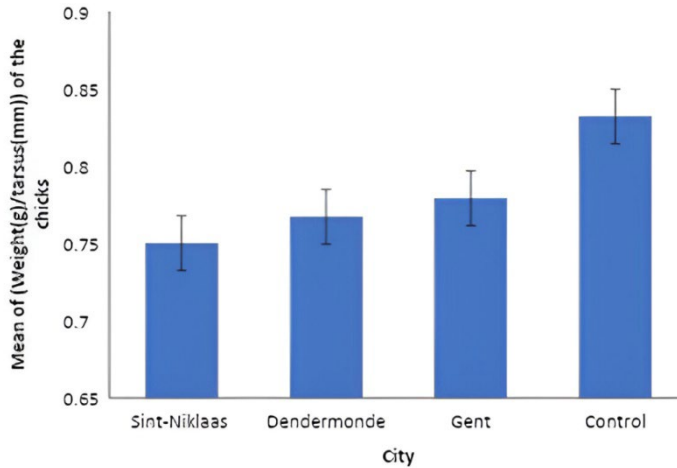


Figure 8. Condition of Great Tit chicks (Weight(g)/tarsus(mm)) of the chicks at day 15 in different cities and the control forest area.

DISCUSSION

Based on our results, we conclude that both House Sparrows (*Passer domesticus*) and Great Tits (*Passer major*) can be considered indicator species for urban habitats. For Great Tits, urban environments appear to function as an *ecological trap*.

In human-dominated landscapes, (semi-)natural habitats are typically embedded within a matrix of unsuitable environments. For territorial birds like Great Tits and House Sparrows, this spatial configuration results in longer foraging times to collect enough food for their nestlings, which ultimately leads to poorer nestling condition. Havlick et al. (2022) similarly found that urban House Sparrows had larger foraging ranges and longer foraging distances during the breeding season compared to rural birds. For both species, the

surrounding landscape composition directly influences food availability and the cost of its acquisition.

Urban environments may also provide novel ecological opportunities, such as an (over)abundance of food in winter from garden feeders. This may lead to improved body condition in early spring, which could explain why urban Great Tits initiate breeding earlier and lay, on average, one more egg than their forest counterparts. However, due to suboptimal food availability—particularly low insect abundance—during the breeding season, these birds are unable to raise their chicks optimally (De Satgé et al., 2019).

The "**LOB Cities Model**" offers a valuable framework for enhancing urban biodiversity, but its reach often does not extend into the dense cores of cities. Many urban centers remain significant biodiversity challenges, as they offer limited space and opportunity for greening interventions. Strategies to increase urban biodiversity in these areas can be grouped into five key categories:

1. **Green roofs** – Beneficial for managing rainwater and providing habitat for birds.
2. **Vertical greenery** – Crucial in dense urban streets where trees and shrubs cannot easily grow.
3. **Dense shrubs** – Offer birds essential cover for roosting, shelter, and breeding.
4. **Hedges** – Versatile and suitable for placement in a variety of locations such as parking lots, between buildings, and alongside roads and pathways.
5. **Herbaceous borders** – Can be planted in underused or 'lost' spaces and integrated into lawn mosaics.

Much remains to be done to shift our perspective and view cities as ecosystems that provide essential ecosystem services. Urban planners can play a critical role by incorporating biodiversity requirements into zoning regulations, architectural guidelines, and development plans.

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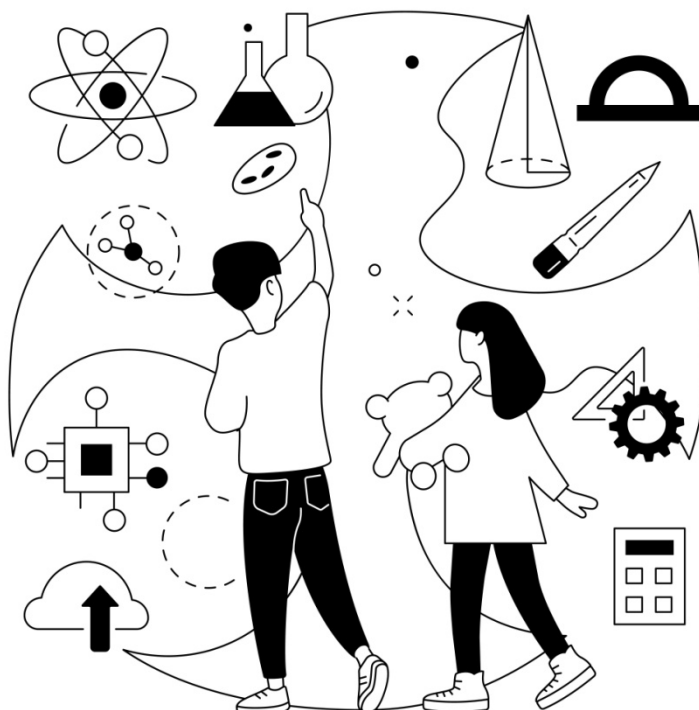
How to make science from the playground using the House Sparrow as a model?

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How to make science from the playground using the House Sparrow as a model?

Francisco ATIÉNZA

ABSTRACT

The House Sparrow (*Passer domesticus*) requires holes or roof tiles for breeding. However, modern architectural trends—often lacking tile roofs—are contributing to the global decline of the species. Although a vast number of studies have been published on this bird, it is rare to conduct scientific research using data collected from a school playground. In one such initiative, schoolchildren and their teacher launched a scientific project using this species as a biological model. Approximately 20 pairs breed and feed within the school grounds each year, and many other sparrows come from nearby detached houses. Our goals were as follows: To teach students how to collect standardized data and enter it for further analysis, to collaborate with scientific institutions to study behavioral, physiological, and survival aspects, and to promote citizen science. Data are collected using a homemade trap and mist nets. Captured individuals are color-ringed, and biometric and condition measurements are recorded. Recoveries are carried out using two techniques: The first involves students spotting color-banded birds in the playground and on rooftops using 60x terrestrial telescopes (high school students). The second involves reviewing footage from a 24-hour Wi-Fi camera (primary school students). To date, 35 individuals (22 males and 13 females) have been trapped. One recovery was made using the first technique, and eight using the second.

KEYWORDS

Colour-ring, House Sparrow, school playground, science, schoolchildren, nature reconnection

INTRODUCTION

Children today, particularly those in urban areas near natural environments, are more disconnected from wildlife than ever before, while simultaneously becoming more connected to technology in their daily lives. In fact, new technologies play a significant role in people's lives and are increasingly being used as a means for citizens to connect with nature (Fathima & Isaath, 2022). However, this modern form of nature connection may contribute to a large-scale “extinction of experience” with the natural world—referring to the loss of direct human–nature interactions, as described by Soga and Gaston (2016; see also Gaston & Soga, 2020). Therefore, if we aim to change this trend among schoolchildren, it is advisable to promote scientific engagement not only in laboratories but also through environmental education integrated into school curricula. This approach holds great potential for raising environmental awareness and helping students acquire the knowledge, skills, values, and experiences needed to take individual and collective action in addressing environmental challenges. Unfortunately, current primary and secondary science curricula often do not sufficiently encourage or support students in developing their ability to explore and investigate nature through direct contact with wildlife and natural environments.

Environmental outdoor activities, including participation in conservation programmes, can be easily scheduled for teachers when they involve short distances from school. The longer the distance, the less frequently students visit natural areas, resulting in fewer opportunities to engage in collaborative projects, collect data, or actively participate in scientific activities. As outdoor learning becomes increasingly challenging for many schools—particularly when visiting wild environments requires transportation such as buses—teachers must consider alternative options that provide comparable potential for learning and involvement in research projects. Urban parks near schools, school gardens, and playgrounds can serve as viable alternatives for connecting schoolchildren with flora, fauna, and, in some cases, scientific exploration. Reconnecting with nature offers significant health benefits and, crucially, ecological advantages, fostering public support for activities

aimed at reversing the global loss of species, including urban species like the House Sparrow.

Thus, identifying the most effective ways to reconnect and engage students with nature is a top priority. Evidence suggests that childhood experiences with nature can positively influence families by promoting wildlife conservation and fostering pro-environmental attitudes and behaviors in adulthood (Kidd and Kidd, 1996). To cultivate such attitudes in children, fundamental knowledge of plant and animal identification is crucial for understanding and conserving biodiversity (Lindemann-Matthies, 2005). Unfortunately, science studies at the secondary level using birds as biological models are scarce, and in Spain, they are virtually nonexistent. Hirschenhauser et al. (2019) demonstrated that participation in scientific projects significantly enhances students' learning, knowledge, and interest in nature. Similarly, Ortega-Lasuen et al. (2023) found that students exposed to a bird-centered curriculum during primary education possess a better understanding of local biodiversity.

Our primary aim is to “combat” the “extinction of experience” and reconnect students with nature through science. Specifically, our project is designed to enhance students' learning and interest in the house sparrow and its biological aspects. We also have the following objectives:

- Teaching students how to collect standardized data and input it for further analysis.
- Collaborating with scientific institutions to study aspects such as behavior, physiological states, and survival.
- Promoting citizen science.

- Providing opportunities for students to observe nature in the schoolyard and distinguish between sexes based on external characteristics.

Conducting this project within the school perimeter offers several advantages: a) Saving time and money, thus preserving valuable teaching time. b) Allowing for frequent visits, which facilitates long-term studies. c) Enhancing attention, as pupils tend to be more focused, learn better, and feel more comfortable in familiar environments compared to novel ones (Rickinson, 2001; Rickinson et al., 2004). d) Fostering awareness, knowledge, and positive attitudes towards local biodiversity when education takes place outside the classroom but within school grounds, as observed by White et al. (2018). e) Supporting mental health in an increasingly urbanized and stressful world.

MATERIAL AND METHODS

Our project is being carried out at San José de la Montaña School, located in Oliva (Valencia), 1 km from the city. The school features a large playground and a small garden within its premises. Birds were trapped during the school timetable using two techniques: (a) mist-nets (Fig. 1), and (b) a 40x40x80 cm wire cage with seeds provided *ad libitum* (Fig. 2). The adult birds caught were sexed, aged (categorized as either young—first calendar year—or at least one calendar year or older, based on plumage characteristics; Svensson, 1992), weighed (using an electronic balance accurate to the nearest 0.1 g), and their tarsus length measured (using a digital caliper accurate to the nearest 0.01 mm). Each adult bird was marked with an individually numbered aluminum ring and colored rings (a three-color code). Superglue was used to seal the colored plastic split-rings, as previous studies (see Bernat-Ponce, 2020) found that sparrows tend to remove the rings with their strong beaks. After all data were collected, the birds were released by students (Fig. 3).

Data were gathered using two methods. First, students aged 15 to 16 years old searched for banded birds either on rooftops or in the school playground (Fig. 4) using binoculars and 20-60x terrestrial telescopes (Fig. 5). Second, a 24-hour WiFi camera (Reolink 2K 3MP model, Fig. 2) continuously recorded all activity of the birds attracted to

the seeds. The camera was positioned to focus on the wire cage in the indoor garden. This setup allowed primary school students to collect data either through live streaming or by analyzing previously recorded videos. When a banded bird was clearly identified on the screen, students noted the corresponding colored rings in their drawings. The gray color indicated the aluminum ring (see Fig. 6).



Figure 1. Mist-nets placed in the indoor garden.



Figure 2. Wire cage with funnel entrances and the 24h wifi camera.



Figure 3. Primary students releasing a House Sparrow female ringed and data taken.



Figure 4. A resight of a male on the roof in the detached house next to the school.



Figure 5. Students searching for banded birds using telescopes and binoculars.

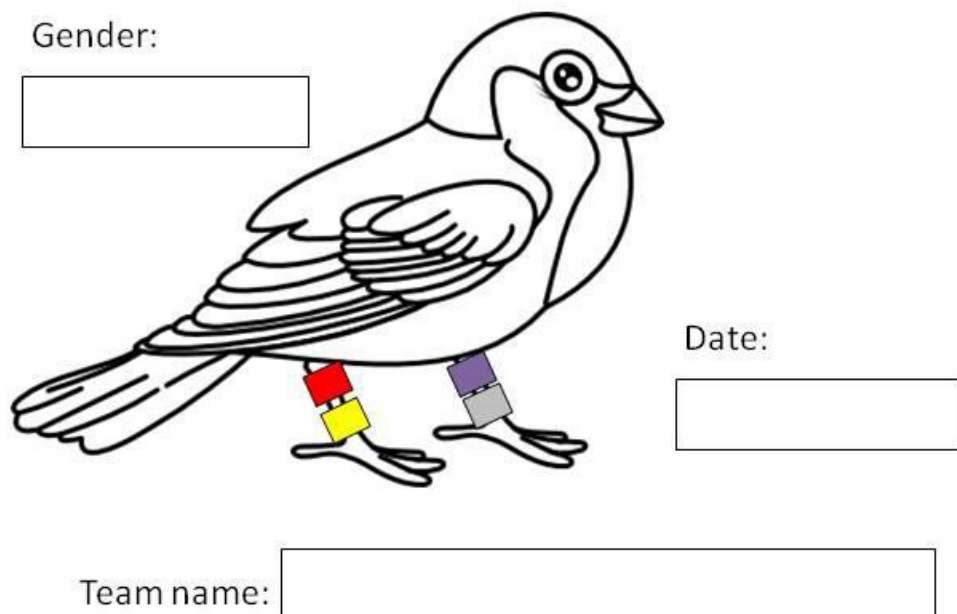


Figure 6. A drawing model coloured by Primary students after playing recorded videos.

RESULTS AND CONCLUSIONS

A total of 35 birds were captured, 22 of which were males and 13 females. Most of them breed within our facilities, and we have observed many unmarked birds in the school playground. To increase the number of birds captured, particularly those breeding in the houses near our school, an additional wire cage trap and a 24-hour WiFi camera were placed in the school garden.

No rats have been detected in the cage while feeding seeds; however, a feral cat visited twice. These feral cats have been safely trapped and relocated to a charity animal center. Once the cage is prepared for trapping birds, it becomes inaccessible to cats. The main issue we identified was a Yellow-legged Gull (*Larus michahellis*), which has been turning some tiles upside down in search of nestlings.

I strongly believe that incorporating this new experience with living animals outside of the classroom represents an extraordinarily promising approach to teaching science. To conclude, I would like to paraphrase Carazo (2024): “Basic research is fundamental. Among other things, it forms the foundation of all applied science. To address the issue of disconnection with the public, we should focus on improving and expanding communication, while placing greater emphasis in schools on the scientific method—on how science works, why it helps us better understand the world around us, and its impact.”

ACKNOWLEDGEMENTS

I would like to thank my colleagues who helped me in preparing the project in order to apply for a two-year funding as well as the schoolchildren who actively participate in different activities. The research and educational innovation project has been funded by Conselleria de Educació, Cultura y Deporte (3200557 PIIE). I’m grateful to Edgar Bernat who encouraged me to show the project that I had recently launched at the 7th Working Group Urban Sparrows Meeting held in Valencia and to continue the research in House Sparrows.

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This volume compiles the key insights and research presented at the **7th Meeting of the Working Group on Urban Sparrows**, held in Valencia in 2023. After several years of anticipation, this international gathering brought together researchers from across Europe and Asia—including participants from countries such as Spain, the Netherlands, Belgium, Finland, and India—to share knowledge and strategies for the conservation of urban bird species.

The book includes both the **abstracts from the meeting** and the **development of six full chapters**, presented as scientific papers. These contributions reflect the latest research on the **House Sparrow (*Passer domesticus*)** and its ecology, alongside studies on other indicator species such as the **Great Tit (*Parus major*)** and the **Tree Sparrow (*Passer montanus*)**.

The book explores topics such as ecology, behavior, threats, and conservation in anthropized environments. It addresses pressing issues including plastic incorporation into nests, population monitoring, breeding success, and comparisons between urban and rural ecosystems.

Beyond serving as a record of the 2023 meeting, this volume provides a valuable reference for current trends and future directions in urban avian research. It is a useful resource for scientists, conservation practitioners, and students interested in the interaction between biodiversity and urbanization.

Published as an **open access edition** by **Publicacions de la Universitat de València** and funded by the **Institut Cavanilles de Biodiversitat i Biologia Evolutiva**, this work aims not only to inform but to encourage continued scientific exchange and concrete actions to safeguard urban wildlife.