UNIVERSITÀ DEGLI STUDI DI PALERMO

From Literature to Acoustic Monitoring: Exploring the Ecology, Conservation, and Landscape Interactions of Sicilian Bats

Mark Massaad

Doctor of Philosophy

January 2024

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Corso di Dottorato in Sistemi Agro-alimentari e Forestali Mediterranei Dipartimento di Scienze Agrarie, Alimentari e Forestali
CiCLO XXXVI

Mark Massaad

Doctor of Philosophy

Tutor: Tommaso La Mantia

Co-tutor: Rafael Da Silviera Bueno

External Co-tutor: Ilham Bentaleb

January 2024

Personal thoughts

If you ever asked me if I imagined myself studying bats, my answer would be **NO.**

Never take anything for granted.

We Learn from our decisions.

Despite all the difficulties and challenges, I am grateful for this decision.

From now on you can call me Batmark

Abstract

Islands are renowned for their unique patterns of bat species assemblage and endemism, yet they are still among the most threatened taxa. Despite being the largest island in the Mediterranean, research on Sicilian bats is still inadequate and scattered. This Ph.D. aims to identify research trends, priorities, and status of research in the Mediterranean and Sicily. According to the procured research gaps, we conducted an acoustic monitoring study to assess the following research gaps: How vegetation structure, landscape scale and climatic variables can influence bat activity and assemblage? And what species are occurring on the least studied small islands around Sicily?

Systematic reviews revealed unbalanced research efforts across geographical areas, a bias in the studied species, a lack of knowledge regarding bats' interaction across natural habitats, and little research targeting small islands around Sicily. Acoustic data demonstrated the presence of 15 bat species/phonic among 10,957 calls recorded during 262 nights across Ficuzza natural habitats. Landscape factors such as proximity to unpaved roads, anthropogenic structures, and dung density significantly influenced bat activity. Bats responded differently depending on their foraging guild groups. Bats exhibited varied responses based on foraging guilds, with different nightly activity patterns observed. Through sporadic acoustic monitoring, we also demonstrate the importance of the small islands around Sicily. We reported new species for the first time, including *Nyctalus* spp. in the Aeolian islands, *H. savii* and *P. pipistrellus* in the Pelagie Islands (Lampedusa), and *T. teniotis* in Pantelleria. This thesis demonstrates the importance of systematic and acoustic as evidence-based approaches to identify, foster, and fill research gaps. Further studies are still needed to assess bat distribution, abundance, and their interaction across different ecosystem and habitats for long-term conservation.

Publications

Published

- **Massaad, M.,** da Silveira Bueno, R., Bentaleb, I., La Mantia, T., 2023. Bats of Sicily: historical evidence, current knowledge, research biases and trends. Natural History Sciences, 10(2).

- **Massaad, M**., Bueno, R. D. S., Bentaleb, I., La Mantia, T., 2022. Priorities and gaps in the Mediterranean bat research evidence: a systematic review for the early twenty-first century. Hystrix, 33(2).

- Chen, K., **Massaad, M.,** De Lima, R., Rainho, A., Rocha, R., 2023. First observation of *Lethocerus cordofanus* (Hemiptera: Belostomatidae) preying on *Afronycteris nanus* (Chiroptera: Vespertilionidae). Entomological Science, 26(3).

Under Preparation

- **Massaad, M.,** Bueno, R. D. S., Bentaleb, I., La Mantia, T., Bats in Mediterranean Agroforestry: Exploring activity patterns and spatiotemporal dynamic

- **Massaad, M.,** Bueno, R. D. S., Bentaleb, I., La Mantia, T., Newly recorded species across Sicilian archipelago: A sporadic acoustic monitoring of bats across the Sicilian small island

- Fernandez, G., **Massaad, M.,** Rainho, A., Island Trees in a Sea of Rice: Unveiling the influence of isolated trees on insectivorous bats and people in Guinea-Bissau.

Acknowledgment

I want to express my deepest gratitude to all those who have been instrumental in making my doctoral journey a transformative experience.

First and foremost, I am grateful to the University of Palermo for funding this project, which significantly contributed to the success of my doctoral journey. Their support underscores the university's commitment to research excellence.

I thank my supervisor Prof. Tommaso La Mantia and my co-supervisors Dr. Rafael da Silviera Bueno and Prof. Ilham Bentaleb for their scientific guidance, advice, help and for being there when needed during this period. I want to also express my sincere appreciation for our PhD coordinator Prof. Vincenzo Bagarello for his support during this period.

I would also like to thank all the colleagues and professors that I met during my stay at the university. A special thanks to my friends and colleagues Dario Scuderi, Antonino Ioppolo, Angelo, Valeria, Roberto, Alessandro, Stefano, Danielle, Carmelo.

I extend my heartfelt thanks to the friends I made in Palermo. Your camaraderie and warmth turned a distant place into a home away from home, and I am truly grateful for the shared moments of joy and support: Luis, Elisavet, Victor, Salvo, Eleonor, Lotte, Ana, Kristina and all other friends.

To my parents Michel and Katia, and my siblings Maroun, Raymond, Elie, and Micha who stood unwaveringly by my side throughout this challenging expedition, I owe a debt of gratitude. Your encouragement and belief in me fueled my perseverance. Thanks to my friends in Lebanon; Joe Merheb, Charbel Fadel, and Marc Habchy for your continuous support.

Special thanks to Prof. Ana Rainho for her mentorship and for warming welcoming during my time in Guinea and Portugal. I am also thankful to Ricardo Rocha (Oxford University), and Ana Filipa Palmeirim (CIBIO) for their invaluable guidance during my time in Guinea-Bissau.

Throughout my PhD journey, I have been fortunate to have a network of colleagues, mentors, and friends who have enriched my academic pursuits. I am grateful for the collaborative spirit and the shared pursuit of knowledge that characterized our interactions. In conclusion, this dissertation is a testament to the collective efforts of those who have supported, inspired, and guided me. I am profoundly thankful for learning and growing in such an esteemed company.

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Chapter 1

General Introduction

General status of insular bat research through bibliometric analysis

Insular bats are commonly perceived as a highly threatened species (Jones et al., 2009). They are facing several challenges arising from environmental and anthropogenic pressures, as well as from insufficient or scattered data. A potential strategy to address these challenges involves reviewing existing data through systematic, literature review and bibliometric studies. This approach can offer a comprehensive exploration of existing studies and can provide solid baseline for future research and conservation strategies. Bibliometric analysis is considered a solid approach to understand the current research status and trends (Cao et al., 2022). In this context, we performed bibliometric analysis using the Scopus database, employing the following keywords "bat" OR "chiroptera" AND "island". We obtained a total of 605 documents published between1953 and 2023 with an average annual growth rate of 4.54%. A total of 558 documents were indexed as “article”, 32 documents as “review”, seven as “book chapter” and the rest were considered as conference papers, letters, and notes. An average of 12.6 documents were published per year. Most of the documents were published in English followed by Spanish, French, and German language. The highest number of documents was published in 2017 and 2021, with 36 documents each, respectively.

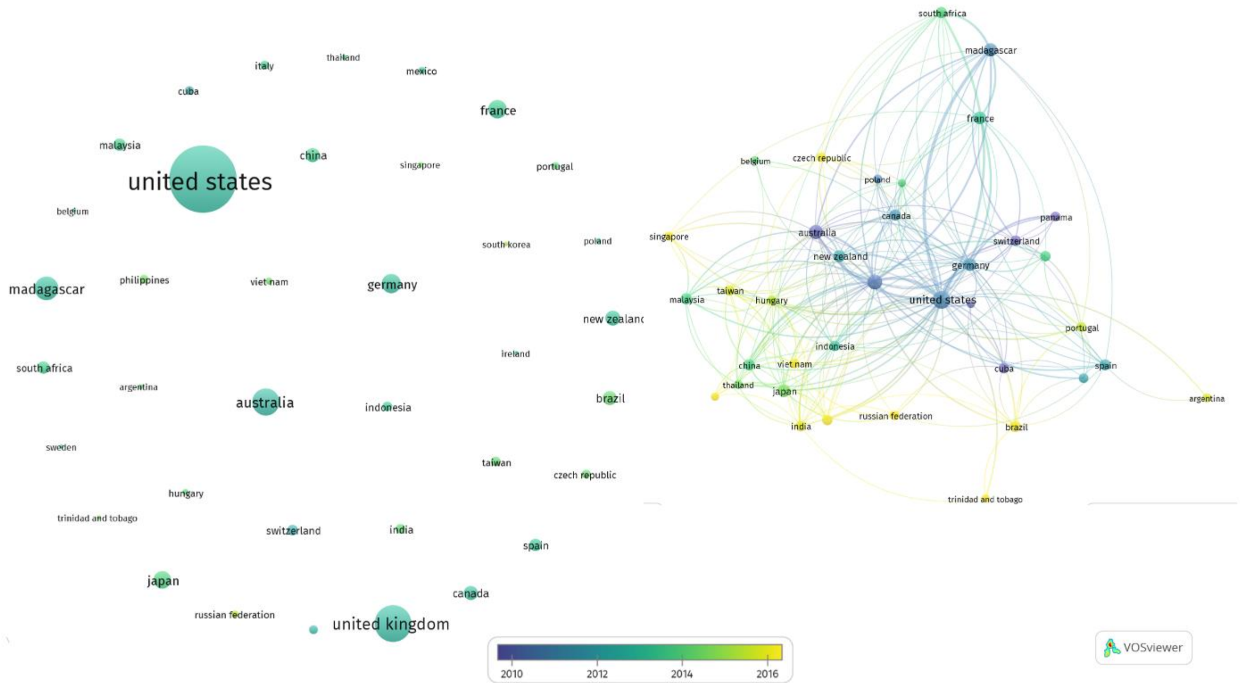


Figure 1. Analysis of co-authorship and publications across nations. The size of the circles reflects the relative frequency, while the colours denote the year when co-authorship was established.

87 countries contributed to the publication of research concerning insular bats. United States of America had the highest number of publications overall with 547 documents, followed by Australia (225), the United Kingdom (201), France (164), Japan (156), and China (138). Brazil and Madagascar had the same number of publications with 113 documents each (**Figure 1**, **Figure 2**)

A strong collaborative link was established between the countries and the continent. UK and USA collaboration stands out as the strongest collaborative liaison with 24 documents. Other intercontinental American collaboration was found between France-USA (16) followed by USA-Australia (15), Australia-UK (14), UK-South Africa (14), France-Madagascar (13), UK-Madagascar (12), Germany-Panama (12), and UK-China (11). On the other hand, some strong intracontinental collaborations were observed. In Africa, Madagascar-South Africa contributed to the publication of 18 publications. In the Oceanian, nine publications were published by the collaboration of authors from Australia and New Zeeland. In Asia, a total of 9 publications were published following collaborative work between Chinese and Japanese institutions.

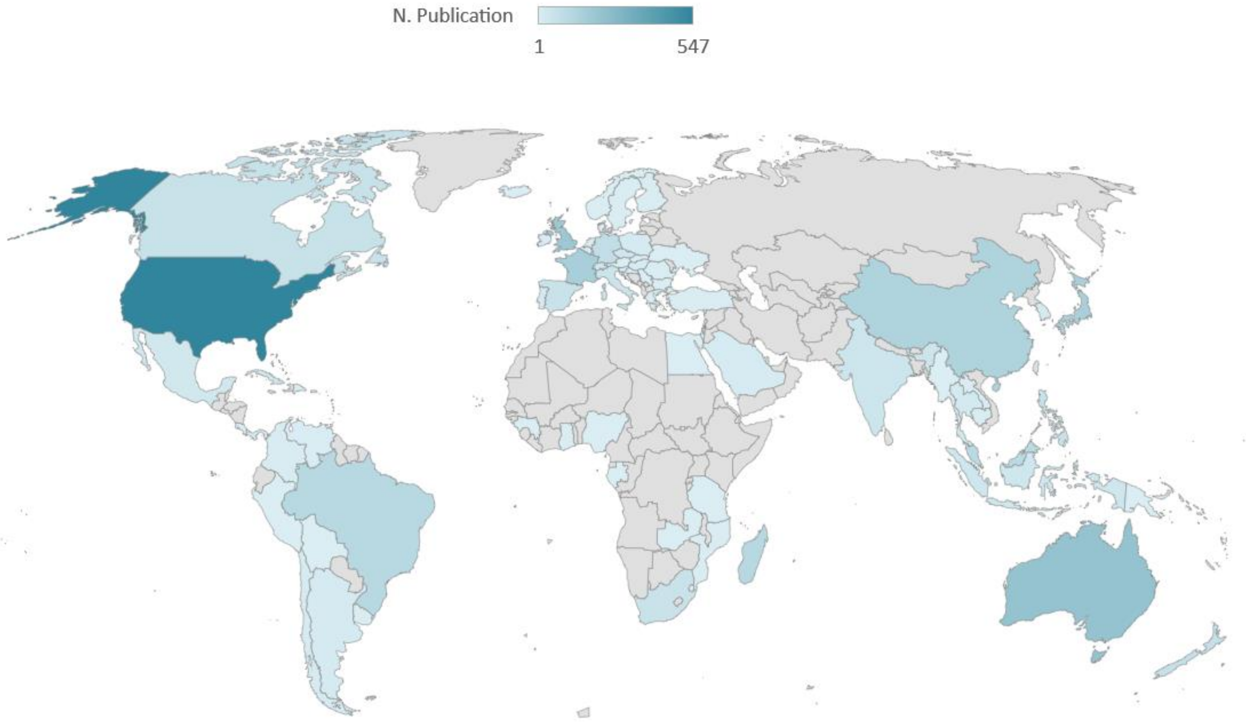


Figure 2. Number of publications per country over the last 70 years.

As to the number of citations, the highest number of citations was scored by the USA documents with a total of 2772 citations followed by the UK (1431), Germany (762), Malaysia (744), and Australia (61). It was also relevant that research targeting health, virus and ecology scope were the most cited articles, here we name the top cited articles: The article with the highest citations titled "Isolation of Nipah virus from Malaysian Island flying foxes" (Chua et al., 2002) till date the article was cited 488 times, followed by "Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007" by Leroy et al. (2009) cited 397 times, "A review of the global conservation status of bats" by Mickleburgh et al. (2002) cited 328 times, "Predicting biodiversity change and averting collapse in agricultural landscapes" by Mendenhall et al. (2014) with a total number of 238 citations and "Flying foxes cease to function as seed dispersers long before they become rare" by McConkey et al. (2006) cited 214 times.

Ecological and economic role of bats

Bats are the second-largest order of mammals with over 1,460 species. They are found in all environments across all continents, except Antarctica (Wilson et al., 2019). Renowned for their ecological role and contributions, bats are recognized as key pollinators, seed dispersals, and natural insect predators. Bats exhibit exceptional insect control capabilities, with a single bat consuming up to 100% of its body mass in insects nightly, most of which are pests (Kunz et al., 2011). Insectivorous bats emerge as principal nocturnal predators of insects, including major agricultural and forest pests (Maine and Boyles, 2015; Federico et al., 2008). The economic value of bats in pest control services is estimated by billions of dollars, particularly in non-conventional agriculture systems (Maine and Boyles, 2015; Boyles et al., 2011; Cleveland et al., 2006). Bats can contribute directly by mitigating crop damage or indirectly by decreasing reliance on insecticides.

Studies reveal that bat pest control services vary across plots, influenced by plot location, size, and crop type (Ancillotto et al., 2024). In the last years, several studies have quantified the economic role of bats across different types of habitats and agricultural crops. For instance, Cleveland et al. (2006) estimate the economic value of bats in cotton fields in south-central Texas at approximately $741,000 per year. Always in cotton fields, bats save up to 63 million US dollars as a natural control for cotton bollworms (Kolkert et al., 2021). Similar assessments highlight the economic importance of bats in vineyards, with estimates ranging from 188 to 248$ per hectare per year (Charbonnier et al., 2021). Because of bats, wine growers in Chile save up to 7% of their annual production (Rodríguez-San Pedro et al., 2020). The economic contribution extends to other crops, with insectivorous bats providing ecosystem services valued at 613$ per hectare, suppressing pest species such as stinkbugs (Taylor et al., 2018). In Thailand, the predation of white-backed plant hoppers (*Sogathella furcifera*) by wrinkle-lipped bats (*Tadarida plicata*) prevents rice crop losses valued at over $1.2 million annually (Wanger et al., 2014). In corn fields, bats make a direct contribution estimated at approximately 788$ per hectare by reducing the requirement for pesticides and protecting the crop from damage (Puig-Montserrat et al., 2015; McCracken et al., 2012). In Italy, Ancillotto et al. (2024) estimated bats' economic contribution to apple orchards at around 550 euros/ha/year.

The critical role of bats in pest suppression is exemplified in different studies, such as the predation of cotton bollworm moths (*Helicoverpa zea*) by Brazilian free-tailed bats (*Tadarida brasiliensis*) (McCracken et al., 2012). A recent study by Weier et al. (2018) investigated insect pest consumption by bats in macadamia orchards, revealing variations in pest consumption among different bat species based on dietary preferences and foraging techniques. The foraging behavior of bats, once considered somewhat vague, appears to be relatively selective and opportunistic during insect predation (Heim et al., 2017). This adaptability enables bats to actively participate in the natural mechanism of pest suppression during insect outbreaks (Davidai et al., 2015; McCracken et al., 2012).

Besides their role as insect suppressors in agricultural systems, bats have been demonstrated to play an important role in suppressing forest pests. Some studies highlighted the role of bats in the agroforestry ecosystems (Novella-Fernandez al., 2022; Korine et al., 2020). Ancillotto et al. (2022) found that two forest-dwelling bats species *Plecotus auritus* and *Barbastella barbastellus* feed on a wide range of forest insect pests in beech forests in central of Italy. These species are also described to efficiently control insect outbreaks of forest moths at the larval stage (Blažek et al., 2021). In pine plantations, forest bats showed an efficient control of pine defoliator moths (Charbonnier et al., 2014). In recent findings, bats have been discovered to play a crucial role in reducing both insect density and defoliation in oak forests in the United States (Beilke and O’Keefe, 2023).

In conclusion, bats play a crucial role in agricultural and forest pest control, offering economic benefits and contributing to sustainable farming practices and natural systems. Their selective foraging behavior, diverse dietary preferences, and adaptability make them a keystone element in maintaining ecological balance and reducing the reliance on chemical insecticides. The economic value of bats in pest control underscores the importance of conservation efforts to ensure their continued contribution to ecosystems worldwide.

Bats habitat selection

While caves may be the first habitats that come to mind when thinking about bats, numerous species favor forests, agricultural areas, shrublands and urban areas as their primary habitat (Dietz et al., 2018; Russo et al., 2016). Selected habitats should provide fundamental opportunities for bats to roost and forage, offering a variety of cavity forms that cater to a range of thermal conditions suitable for different bat species (Dietz and Hörig, 2011). They support various aspects of bat's life cycle from foraging, roosting, reproduction, spring emergence, fall swarming, and hibernation (Fuentes-Montemayor et al., 2017). In general, habitat preference varies among species, geographical locations, and life cycle stages (Silvis et al., 2016).

Several factors contribute to the success of bats roosting sites, including the availability of insect prey, the efficiency of bat foraging, and microhabitat features (Carr et al., 2019; Dietz et al., 2018; Tillon et al., 2018). In the last years studies related to bat activity and habitats compositions and structure have increased. In forest areas, bats, being more selective than other mammals, often show a preference for larger and older trees for roosting. This preference is attributed to the increased availability of microhabitats and a greater abundance of prey insects. Moreover, some bat species tend to forage more in unmanaged areas where a higher abundance of standing deadwood can be found (Fuentes-Montemayor et al., 2013). In the Mediterranean forest, bat roosts are primarily dependent on tree types and their features, microhabitats availability, age, slopes, but also on water availability (Regnery, 2013), and anthropogenic disturbance (Rachwald et al., 2021). In the UK, Boughey (2011) showed that forest bats, including *Barbastella barbastellus*, rely mostly on semi-natural broadleaved woodlands (Dietz et al., 2018; Zeale et al., 2012). These woodlands serve as keystone resources for many species (Mendes et al., 2017). In urban areas, some bat species roost in human structure or urban parks, their roosting is generally favored by tree cover networks which may improve the resilience of some bat populations to urbanization (Gili et al., 2020; Russo and Ancillotto, 2015). In agriculture areas, many European bat species can highly benefit from the presence of linear features including hedgerows and grassy waysides (Finch et al., 2020). These features have been shown to increase functional connectivity and permeability into the environment at a landscape scale, thus reducing the 'barrier effect' caused by other features such as streetlights and roads. Bats act as important indicators of structural and functional changes across habitats in general (Kalda et al., 2015; Jones et al., 2009). Hence, understanding how species interact with their roosting and foraging habitats is essential for future conservation and management decisions. Unfortunately, in recent decades, natural habitats have faced unfavorable threatening conditions, including extensive timber production, intensive grazing, wildfires and urbanization. These factors are posing significant threats to natural ecosystems and directly impact the populations of bats, which are highly dependent on these habitats as their primary roost and foraging habitats.

Foraging behavior

Natural habitats serve not only as crucial roosting sites but also play a significant role as foraging areas for bats. Structural and spatial features including the presence of water, gaps, open spaces, habitat diversity, and insect abundance can contribute to the favorability of bat-foraging activity. These factors and habitat characteristics may promote foraging activity and abundance of certain bat species but also act as limiting factors for other species. Foraging activity is not solely influenced by biotic and abiotic factors but also ecomorphological traits of bat species play a significant role in shaping their foraging behavior (Denzinger and Schnitzler, 2013). Given the substantial diversity in echolocation calls, wing morphology, tail form, ear characteristics, and call structure, bats can be classified into three primary foraging guild groups (Denzinger and Schnitzler, 2013; Neuweiler, 1984). This classification helps in understanding the different foraging strategies employed by different bat species within various ecosystems and habitats.

Closed space foragers:

- These bats, often small, slow-moving, and highly maneuverable, employ broadband calls with limited duration.
- They forage in cluttered habitats with high understory and within narrow forest gaps.
- Strategies include flutter detecting mode, passive gleaning mode, and active gleaning mode, using CF-FM signals.

Edge space foragers:

- Bats foraging in edge spaces utilize high-intensity echolocation calls with a combination of narrowband and broadband calls.
- Foraging occurs near the edges of forests, agricultural lands, vegetation, woodland openings, or over land and water surfaces.
- Two foraging modes include trawling (in calm water surfaces) and aerial (in cluttered backgrounds).

Open space foragers:

- Larger, faster bats with lower maneuverability fly and forage above the forest canopy, and open areas.

- Long-duration, low-frequency, narrowband calls are used for echolocation.
- Bats foraging in open space use the aerial mode to capture prey flying far from background targets.

Acoustic monitoring

In recent years, the utilization of sound recording, and acoustic monitoring has become increasingly employed in ecological research (Browning et al., 2017). Acoustic monitoring is currently used as a pioneer tool in unravelling various aspects of species behavior, including communication, foraging activities, species ranges, and identification. This method is widely used for studying bats, providing a robust approach to monitor bat activity, foraging behavior, species distribution, and assess habitat quality based on their activity (Whitby et al., 2014; Frick, 2013).

While acoustic monitoring enables a balanced sampling design, it may exhibit a bias towards high-intensity bat species, which may lead to a potential overlooking of species with lower echolocation intensity. Another limitation occurs, as this method is still considered highly expensive, posing challenges to its widespread implementation in research, especially in the south hemisphere of the world.

On a positive note, recent advancements by Andrew Hill and Peter Prince in 2018 and 2019 have introduced a solution in the form of a low-cost acoustic detector also known as “AudioMoth”. Priced at around 100 euros, the “AudioMoth” is equipped with a built-in microphone, is open-source, and programmable. It is a full-spectrum device with diverse configurations and a sample rate of up to 384 kHz. This device has demonstrated efficiency and has been successfully employed in various bat studies worldwide, including Madeira, Portugal (Ferreira et al., 2022), China (López-Bosch et al., 2022), and Tanzania (Katunzi et al., 2021).

Why bats of Sicily?

Bats are present in Sicilian folklore, though typically portrayed in a negative context. According to Pitrè (1889), one of the greatest Sicilian ethnologists of the nineteenth century, and other contemporary sources, bats are believed to be created by devils. Bats are also thought to host the souls of people who died violently (suicides, tragedy death) (Pitrè, 1889). Such perceptions in particular those associated with the evil spirits and witches are believed to be influenced by Christian beliefs (Sieradzki and Mikkola, 2022). This deeming is still illustrated in some churches in Sicily, such as Chiesa di Gesu-Palermo (

Figure 3). Another tidbit, Pitre (1889) holds that the bat is an old mouse whose wings grow when it can no longer eat. For instance, in Messina, bats were socially notorious for sleeping for six months. Another form of the presence of bats in Sicilian culture and Folklore brings us back to the etymology and the origin of the names of the European free-tailed bat species *Tadarida teniotis*. It's believed that the word *Tadarida*: is derived from the Sicilian and Calabrian dialectic word tadarida (with variations like *taddarita, taddarida, tallarita, tallarida and taddrarita)* which refers to bat regardless of species. The term *taddarita* has become adapted following the descriptions of species specimen catch in Sicily by Rafinesque (Riccucci, 2015). This historical human-bat association makes bats a prevalent species to study across the islands.

Figure 3. An early 18th-century sculpture of a devil with bat wings engraved on the marble column of Palermo's Chiesa di Gesu (Photo credit: Ralf Steinberger).

Study aims and thesis outline

Despite insular bats being one of the most threatened population species, our understanding of these bat’s distribution, interactions, and research status remains limited. This limitation is generally justified by the difficulties associated with researching nocturnal flying animals and the scarcity of long-term funds.

Here, we intended to integrate multiple approaches to firstly identify the research gaps in terms of biased trends and priorities on the Mediterranean scale. Following a similar approach, our objective was to assess the research status of bats in Sicily, with the aim of identifying the main research gaps including geographical regions, habitats, and species biases.

Considering the obtained results, we initiated a field study to address and fill part of the identified gaps. Firstly, we investigated the interactions between bats and vegetation, landscape, and climatic factors, identifying the main driving factors affecting bat activity and distribution. Secondly, to further explore unstudied areas, we sporadically monitored certain islands around Sicily that had received little research efforts and attention in the last decades.

In Chapter 2, we identified the main threatening factors, population trends, species priorities, and understand ongoing research priorities on the Mediterranean scale. We also employed statistical analysis to assess the impact of different types of habitats and land management on bat activity and species richness based on 18 empirical studies.

In Chapter 3, we leveraged all existing evidence and available literature on Sicilian bats to obtain a better insight of the current research status, priorities, and gaps. Additionally, we provided an updated checklist of Sicilian bats.

In Chapter 4, we investigated the influence of landscape structure across different habitats on bat assemblage and foraging activities, assessed the habitat preferences of the species, and tried to highlight the impact of environmental factors on bats' nightly activity over the season and across habitats.

Finally, in Chapter 5, we provided a comprehensive overview of bat species distribution across six distinct islands surrounding Sicily, achieved through sporadic acoustic monitoring.

Chapter 2

Priorities and gaps in Mediterranean bat research evidence: a systematic review for the early twenty-first century

An adapted version of this chapter has been published in:

Massaad, M., Bueno, R. D. S., Bentaleb, I., and La Mantia, T. (2022). Priorities and gaps in the Mediterranean bat research evidence: a systematic review for the early twenty-first century. *Hystrix*, *33*(2) Click here.

Abstract

Bat conservation is one of the top global concerns for research today; however, conservation efforts may still be limited and impotent due to inadequacy and scarcity of data. Hence, identifying research trends, threatening factors, species status, and geographical priorities is an essential tool for future conservation attempts. Here we conduct a comprehensive systematic review to identify current research priorities, trends, general patterns, and gaps regarding Mediterranean bat research. A total of 97 studies were found in the years spanning between 2000 and 2021. There were 18 studies with sufficient data for qualitative statistical analysis to understand the influence of habitats and land management on bat activity and species richness. A yearly average of 4.6 articles was published, with a slight increase post-2010. Out of 61 identified species, 21% of species are threatened. Approximately, 65 % of studies were conducted in the Mediterranean European region, primarily in Spain (29 %), Italy (15.5 %), and Portugal (10.3 %), primarily focusing on forest habitats (38%). We found that Mediterranean bat species received uneven research efforts, with only 15% of research allocated to threatened bats. Around half of the studies focused on the following bat species; *Pipistrellus pipistrellus*, *Pipistrellus kuhlii*, *Miniopterus schreibersii*, *Rhinolophus hipposideros*, *Pipistrellus pygmaeus*, *Myotis myotis*, and *Rhinolophus ferrumequinum*. Our statistical analysis revealed that riparian areas had higher bat activity than forest and agricultural areas. Bat population responded positively to forest management and organic agriculture practices. To reduce future research misalignment between current local research status and future global conservation priorities, we strongly advocate for urgent and additional collaborative efforts to target under-researched species and areas. Finally, our review will provide a general overview and an objective synthesis of the current status of bats in the Mediterranean and serve as a baseline for further effective research.

Keywords: Bat, Mediterranean basin, conservation, ecology, bat activity, systematic review.

2.1 Introduction

Bats are the world's second-largest mammalian group, with over 1460 species (Mammal Diversity Database, 2021). They provide a wide range of ecological services, including being a natural insect predator for a copious of insects, particularly arthropods (Puig-Montserrat et al., 2020; Williams-Guillén et al., 2016; Boyles et al., 2011), seed dispersals, in addition to being well known as a fruit pollinator for several highly economic valued fruits (Florens et al., 2017). They are also well-recognized as excellent bio-indicators of anthropogenic and environmental changes (Russo et al., 2021). Today, billions of dollars per year is the estimate of the bat's economic contributions (Korine et al., 2020; Puig-Montserrat et al., 2015; Kunz et al., 2011). Despite their numerous benefits to the ecosystem, several bat species are experiencing population decline, and 15% are classified as endangered species (IUCN, 2021). The proportion of threatened bats is expected to rise in many regions, including the Mediterranean basin, where conservation efforts and research are still inadequate and scattered (Voigt and Kingston, 2016; De Paz et al., 2015).

The Mediterranean region supports 61 bat species belonging to eight different families (IUCN, 2021). More than 95% of Mediterranean species are insectivores with at least seven endemic bat species. Most Mediterranean bats rely on the forest and natural habitats (Georgiakakis et al., 2018). According to the International Union for Conservation of Nature (IUCN), numerous bat species are currently experiencing population trend declines on a Mediterranean scale caused by environmental and anthropogenic factors (O'Shea et al. 2016). In this regard, forest loss and fragmentation pose a pervasive threating impact on bat survival (Lison and Calvo, 2014). The overuse of chemicals in agriculture is leading to a decline in insect population, therefore reducing bat food availability (Oliveira et al., 2020). Bats are also affected by urbanization, wind farms, water pollution, roads, and climate change (Bideguren et al., 2019; Russo and Ancillotto, 2015; Amorim et al., 2012). However, some factors such as forest management and agricultural practices' implications on bat activity and population assemblages are still ambiguous and controversial (Froidevaux et al., 2021; Bender et al., 2015). In several cases, bat populations showed positive reactions and benefited from forest management (Law et al., 2016; Russo et al., 2010). Agriculture practices, on the other hand, were discovered to have a mixed impact on bat activity and species richness (Puig-Montserrat et al., 2015).

All these factors, combined with the erratic response of bats, compel us to take urgent actions regarding the ongoing changes that may pose undesirable effects on Mediterranean bat populations (Jung and Threlfall, 2016).

Although the global increase in number of research targeting bats conservation (Frick et al., 2019), research and knowledge about Mediterranean bats are still scattered, making it difficultto fully understand the status of bats. The Mediterranean basin was barely addressed as a discrete region. The most recent report assessing the Mediterranean bats was detailed by the IUCN in 2009. Therefore, identifying the main threating factors, and population trends, in addition to understanding ongoing research priorities, is considered an essential step for bat conservation on a national and regional scale. Intuitively, research efforts vary across species, countries, and thematic areas over the years. Hence, updated information on species based on past and current research is paramount to prioritizing future conservation targets. In this comprehensive review, we synthesize the breadth of our knowledge of Mediterranean bat species and quantify recent information regarding population, species and research trends, knowledge gaps, and threatening factors. Through statistical approach analysis, we also aim to assess the impact of different types of habitats and land management on bat activity and species richness. Supported with previous studies, we anticipate evaluating topics and species priorities over the last 20 years. Finally, this paper will effectively provide an overview of the Mediterranean bat status and serve as a solid directional baseline for future studies with the aim of conservation.

2.2 Materials and Methods

Review data research method

A systematic review was carried out between July 20th and July 30th, 2021 (**Figure 4**), following the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) protocol (Moher et al., 2009). The temporal window of the researched papers was bounded between the years 2000 and 2021. A dataset was created based on the published literature obtained from Web of Science and Scopus using the combination of the following keywords: (Bat* or Chiroptera) AND (Mediterranean or Mediterranean basin). Citations from retrieved papers were also checked to ensure a broader range of dataset patterns. Except for the IUCN report, grey literature, thesis, and technical reports were not included. Following the combination of papers from the research sources, a primary scanning was done by sequentially reviewing the title and the abstract of the article, or the whole article, if necessary. Then, a full-text scanning and evaluation were performed.

A total of 97 articles were included and subjected to full-text scanning. Papers were classified based on their target research habitats: forest (1), agricultural area (2), caves (3), labs (4), riparian area (5), multi-areas (6), urban area (7), Sahara (8) and not indicated (9). Furthermore, each paper was assigned to a specific thematic research topic generally deduced from the paper's aim.

Papers were later standardized and classified into the following five main thematic research topics: 'Conservation', 'Disease', 'Ecology', 'Taxonomy' and 'Species records' (Tab. 1). Also, the following information was extracted from each paper: the year of publication, the author and location of the study, target habitats, threatening factors, studied species, and the methodology applied in each study (e.g. morphology, molecular, acoustic monitoring, isotopic analysis, radio-tracking, observation, and mist netting).

For quantitative analysis, we used a total of 18 studies incorporating empirical acoustic datasets reporting bat activity and species richness. Acoustic data was used as it was the most readily accessible and comparable dataset. All extracted acoustic data, except for Barros et al. (2017), were collected during the summer season. All 18 studies were conducted in at least one of three habitats: forest, riparian, or agricultural. Forest and agriculture studies were divided into two subgroups: managed and unmanaged forests, and organic and traditional agriculture, respectively. Also, for each of the study sites, we extracted the following environmental variables: mean annual precipitation (mm) and mean altitude (m.a.s.l). We obtained environmental data from other publications or weather websites (www.wunderground.com/) when information was missing.

Species status and threats

To assess the current status of bat species in the Mediterranean, we categorized all cited species into subgroups based on the most recent IUCN red list status. All species classified as data deficient (DD), vulnerable (VU), and endangered (EN) were considered 'threatened' species, whereas species identified as least concern (LC) or near threatened (NT) were deemed as 'non-threatened' species. Supplementary information on the species was obtained from the IUCN website, such as family, types of foraging, natural habitats, and their geographical distribution across the basin. Due to the various types of threats mentioned in the papers, threats were grouped into nine major groups based on the IUCN list (**Table 1**). Each threat group comprised several sub-threats. For instance, forest disturbance groups include fires, forest management-logging, and snag cutting.

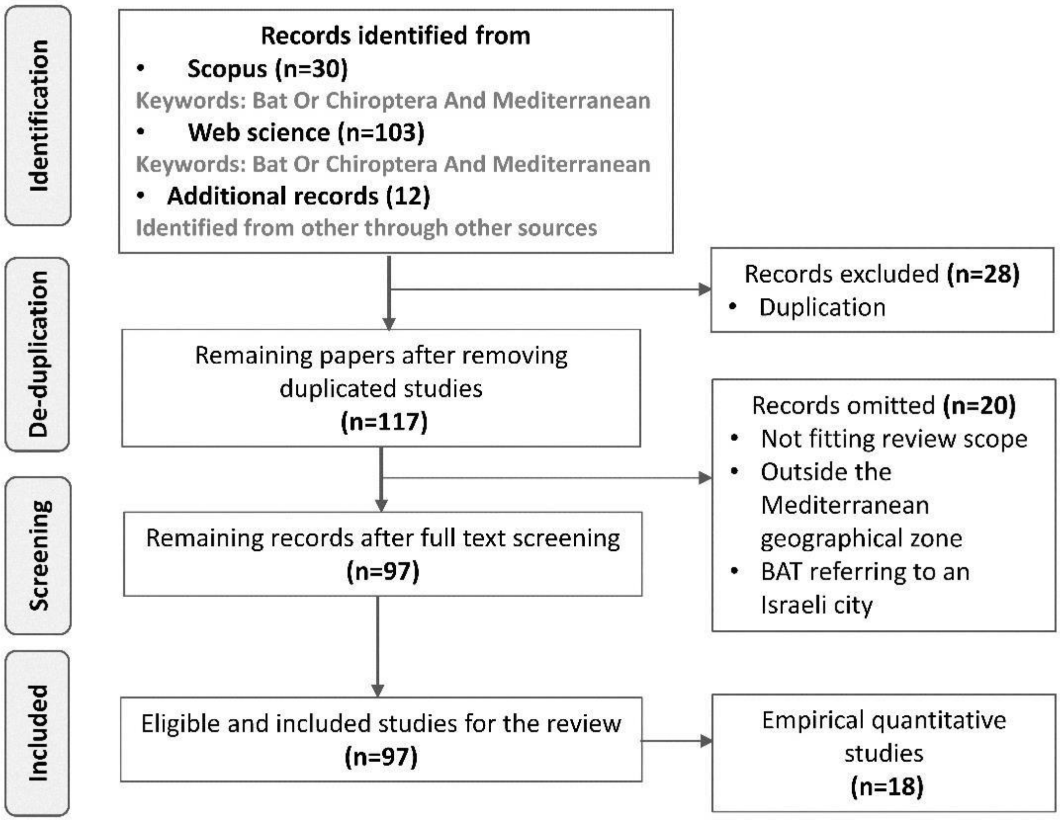


Figure 4. Flowchart of the followed systematic research strategy, displaying the steps taken and the number of included and excluded articles, as well as the criteria used to select the eligible articles for this review (PRISMA by Moher et al., 2009).

Data analysis

The distribution of the number of publications per thematic research groups (Conservation, Ecology, Disease, Taxonomy, and Species records), species taxa, species families, species status (threatened and non-threatened), and per study location (Africa, Europe, and Asia) were tested using Pearson's Chi-square independence test ($\chi 2$). For the quantitative statistical analysis, we extracted the mean bat activity, the number of species identified acoustically, and the sample size, which was the number of study sites for most of the papers. Then, all mean bat activities were standardized into (bat passes/min) and log-transformed to achieve normality. A one-way ANOVA followed by a Tukey Kramer post hoc test was used to determine whether there were any significant differences in bat activity between different types of habitats (agriculture, forest, riparian), as well as between forest management (managed forest, unmanaged forest) and agricultural practices (organic agriculture, conventional agriculture). Generalized Linear Models (GLM) using Poisson distribution were performed to assess the influence of different habitat variables and environmental variables (precipitation and altitude) on species richness and activity. All statistical analysis and plots were carried out with R Studio v4.1.2 using 'lme4' and 'ggplot2' packages (R Core Team 2021).

Table 1. Identified thematic research, threats, and their descriptions (adapted from Preble et al., 2020).

Main Thematic research	Secondary thematic research	Key contents
Conservation	Threats - Conservation plans	Studies with conservation goals. Including studies related to threats, human.bat interactions, conservation strategies…
Disease	Virus - parasites	Involve studies concerning viruses (Lyssavirus), parasites (trombiculids)- insular syndromes
Ecology	Foraging- roosting- ecosystem role - niche modeling- paleoecology- reproductive	Studies dealing with the ecosystem role of bats, habitat selection and foraging sites, bat behavior, migration diet, isotopes, andguano analysis, and niche modeling.
Species records	Checklists- community composition. paleontology- species descriptions	Includes mainly species descriptions in addition and species records and compositions in a specific study area using monitoring techniques (observation, acoustic identifications, mist netting….
Taxonomy	Morphological analysis- phylogenetic- paleontology	Phylogenetic and morphological (wing, anatomical morphology) and histological studies aim to describe bat species taxonomy, in addition to specifying phenotypic and genetic, as well as the differentiation of new cryptic species.
Threats	**Subgroups**	**Descriptions**
Agriculture	Chemical pesticides Agricultural practices	Particularly the overuse of chemical pesticides, which may result in the loss of bat food sources.
Climate change	Temperature change- global warming	It has a considerable impact on roost temperatures and may also affect hibernation periods, as well as resulting in direct mortality in cases of heat waves, intense storms, flooding, and drought.
Data deficiency	lack of information- Under-studied species	Underexplored and poorly studied species may pose direct threats because several species may be on the verge of extinction and are not being adequately protected and monitored due to a lack of information.
Disease	Viruses - bacteria- parasites	Bat zoonotic disease is regarded as a serious threat, particularly in terms of increasing their social dislike as well as directly affecting bat health in cases of parasite and bacterial contamination.
Habitat disturbance	Reconstruction - old building demolition- cave disturbance	This could result in the loss or alteration of foraging habitats and roosts especially present in old buildings.
Forest disturbance	Fires - forest management	Forest disturbances including fires and forest management have a negative impact on bat species richness, community structure, and particularly habitat loss.
Urbanization	Roads - light and noise pollution - reconstruction- wind farms	Causing direct mortality (for instance at wind farm sites), fragmenting habitats with roads, or reducing habitat quality with artificial lightning that many bat species avoid in addition to bat disturbance (during the hibernation phase). by reconstruction practices.
Water availability	Water pollution - water availability	Despite their exceptionally narrow water source requirements, bats are especially vulnerable when water is scarce or polluted.

2.3 Results

Research topic trends over the last 21 years

A total of 97 papers published between 2000 and 2021 were included, with an average of 4.6 articles per year (**Figure 5**). A higher number of papers were published between the year 2010 to 2021 (μ= 5.8 articles/year), compared to an average of 3.4 articles between 2000 and 2010. The highest number of papers published in a single year was nine (in 2007), while only one article was published in 2004 and 2005, respectively. According to the distribution of studies among the five main thematic research topics, the 'Ecology' thematic topic was found to be the most studied with 38.1% of the total articles, followed by 'Species records' (24.7%), 'Taxonomy' (18.5%) and 'Conservation' studies (15.5%), and only three articles (3.1%) focused on 'Disease' studies (**Figure 6**).

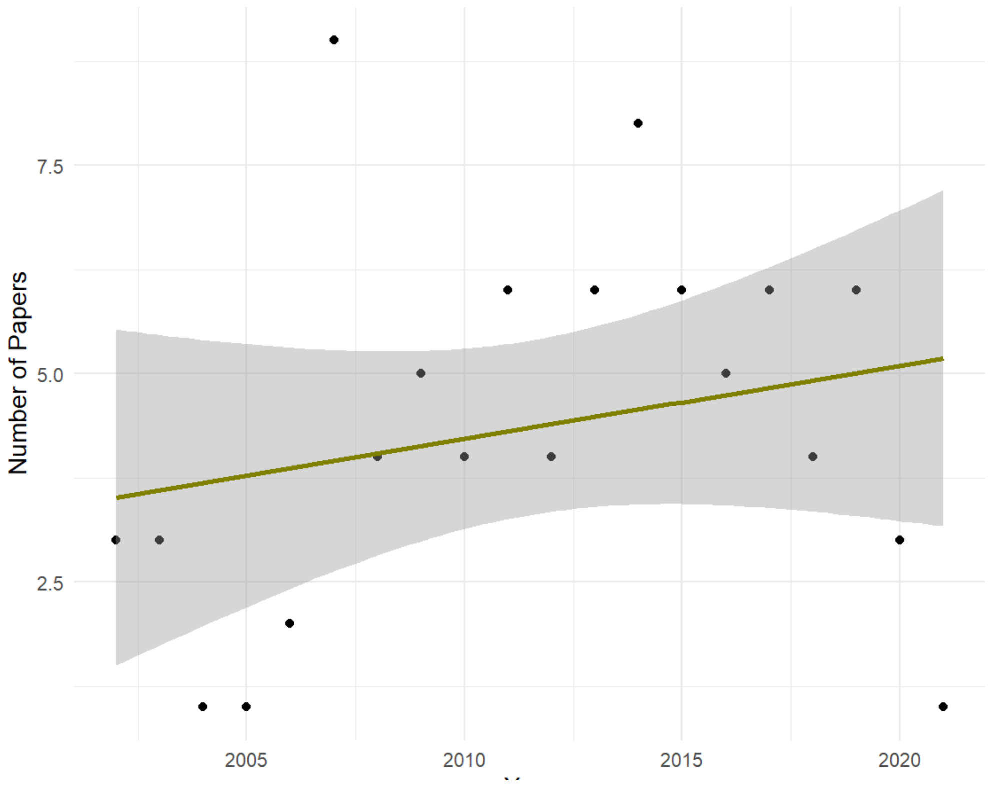


Figure 5. Number of publications over the last 20 years (2000-2021) related to the Mediterranean bats.

Applied methods

Acoustic monitoring was the most used method, with 30 out of 97 articles (**Figure 7**). The 'DNA analyses' was the second most cited method used in 19 studies (19.5 %). 'Morphology' and 'radio-tracking' methods accounted for 17.2% and 15.08 % of the total studies, respectively. While 'excavation' (2.3%), 'isotopes analysis' (3.44%), 'roost observation' (3.44%), and 'modeling technique' (3.44%) were found to be less commonly adapted. Only 4.6% of the total studies applied 'multiple techniques', combining 'morphological' and 'acoustic monitoring' or 'radio-tracking', 'roost observation', 'radio-tracking', and 'DNA analyses'.

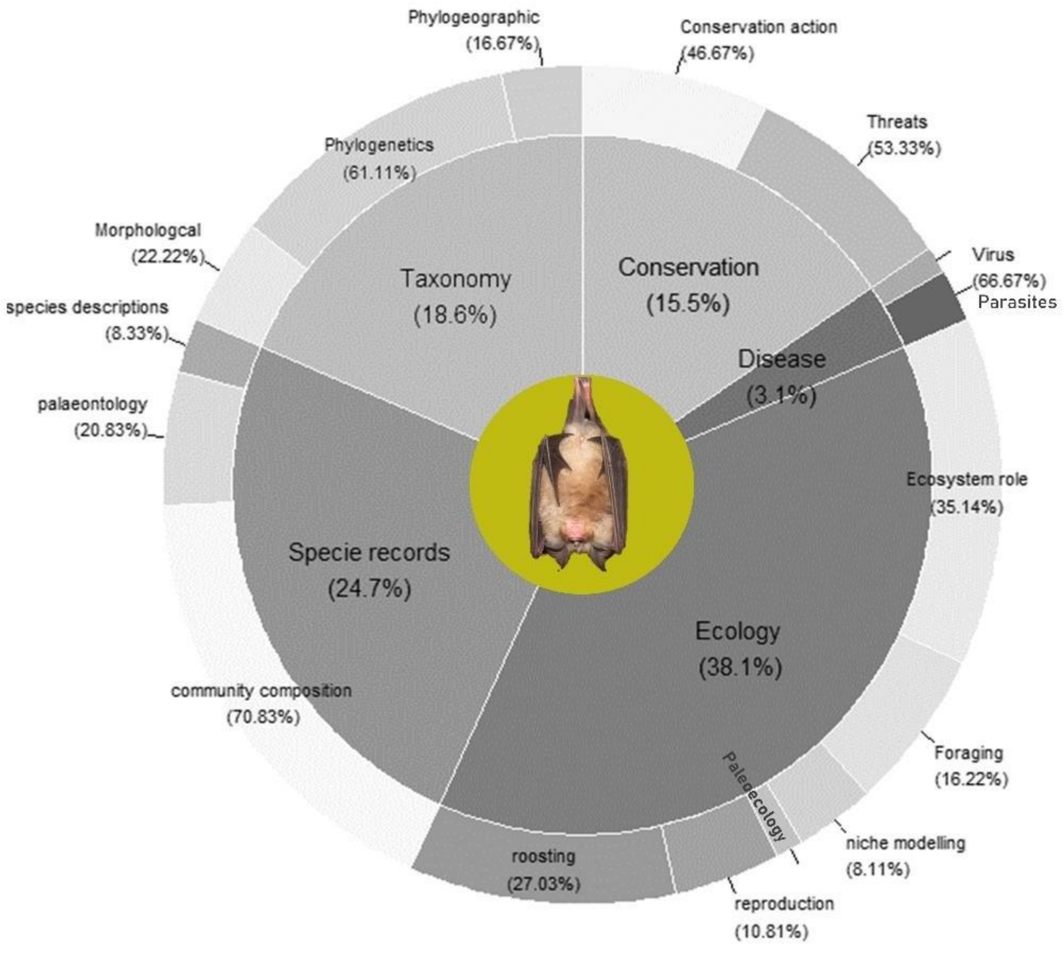


Figure 6. The distribution of publications over the last 20 years according to their primary and secondary thematic research topics.

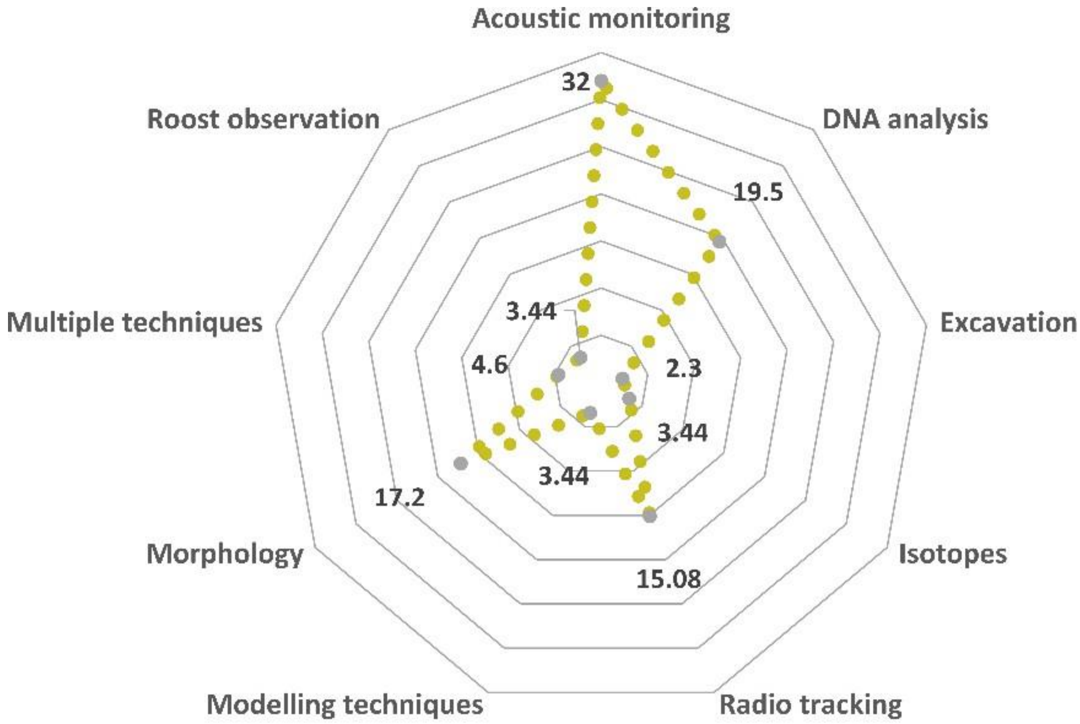


Figure 7. The distribution of the used methods across the 97 studies.

Researched species

A total of 61 bat species belonging to eight families were listed at least once in the 97 analyzed articles. Only one species of bat was identified as frugivorous (*Rousettus aegyptiacus)*, while the rest were insectivorous. A total of 21% of bat species are threatened, with 15% as vulnerable, and 6% with data deficient (**Figure 8**). In terms of the number of species cited per paper, uneven distribution and a significant difference were observed ranging between 1 and 19 ($\chi2$=844.51, d.f= 177, p<0.0001) (excluding the IUCN report and checklists), with a mean average of 3.82 ± 4.25 (mean ± standard deviation) species cited per publication. Vespertilionidae was the most cited family (n=381) (**Table 1**;**Table 2**), followed by the Rhinolophidae (n=67) and Molossidae (n=16). While the rest of the families were rarely quoted, including species belonging to Rhinopomatidae (n=9), Pteropodidae (n=7), Emaballonuridae (n=4), Miniopteridae (n=2), and Hipposideridae (n=2).

On species level, *Pipistrellus pipistrellus*, *Pipistrellus kuhlii*, and *Miniopterus schreibersii* were the most intensively studied species found in 31% of papers, followed by *Rhinolophus hipposideros* (21.8%), *Pipistrellus pygmaeus* (20.6%), *Myotis myotis* (20.6%), and *Rhinolophus ferrumequinum* (19.54%). The remaining species were cited between three to fourteen times, whereas three species were listed only once. Threatened species received less attention, appearing in only 15% of all articles. The remaining 85% of papers focused on non-threatened species, with an average of (8±8.8) articles per species (**Figure 8B**).

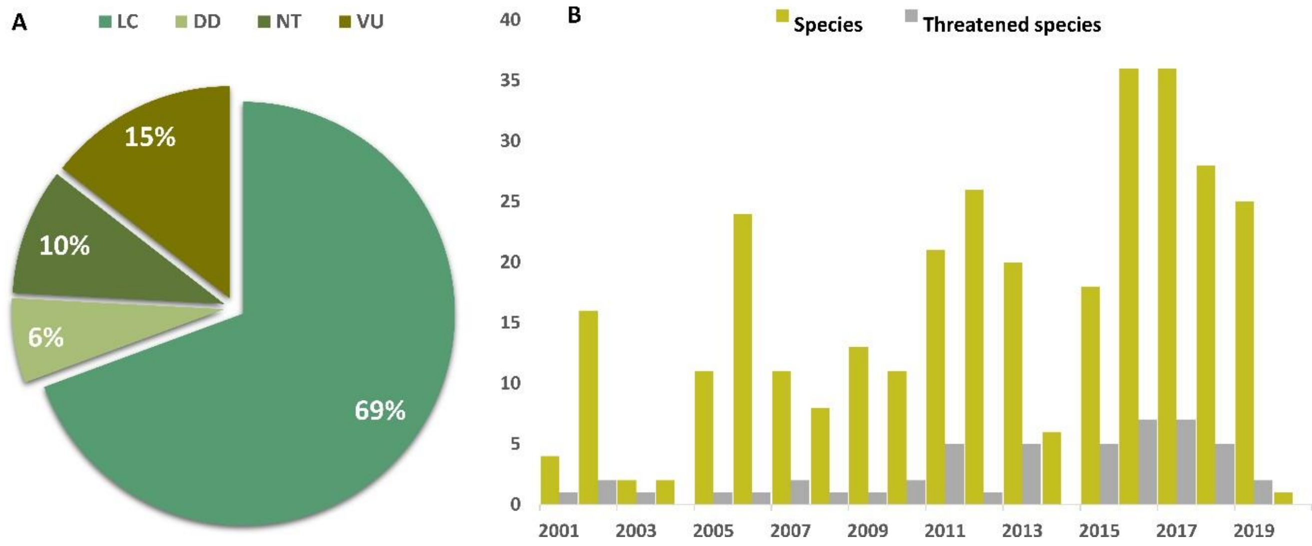


Figure 8. General distribution and trends of Mediterranean bat research between 2000 and 2021 **A:** Pie chart showing the percentage of Mediterranean bats in each red list category based on their conservation status indexed by IUCN (2021); data deficiency (DD), least concern (LC), near threatened(NT), vulnerable (VU) **B:** Number of cited threatened and total species over the years.

Geographical research distribution

Our findings revealed that 97 studies were carried out in 18 different countries spread across three continents (Africa, Asia, and Europe). The number of publications was significantly higher in the Mediterranean European countries than in the Asian and African Mediterranean areas ($\chi 2$=134.125, d.f= 162, $p<0.0001$). Approximately 65% of studies were in the Mediterranean European part (**Figure 9**). Spain, Italy, and Portugal were the most studied countries, with 28, 15, and 10 papers, respectively (**Figure 9**). Mediterranean Asian countries, on the other hand, accounted for only 13.4% of total papers, led primarily by Israel (n=7), followed by Turkey (n=4), Lebanon and Syria one paper each. Nearly 12.7% of the studies were performed across several Mediterranean countries. The Mediterranean African region generated the least number of papers, accounting for only 8.96 % of total publications. Studies focused on nine different target habitats. Around 38% of the total studies (n=36) targeted Mediterranean forests followed by caves (n=15, 15.5 %), agricultural sites (n=11, 11.3 %), and riparian areas (n=9, 9.3 %). Only six studies were conducted in labs and artificial roosts. Sahara was the least researched region with only 3 % of the papers, and two studies did not specify the habitats studied. Notably, 16 papers (16.5%) addressed various habitats, mainly forest, agriculture, and riparian areas.

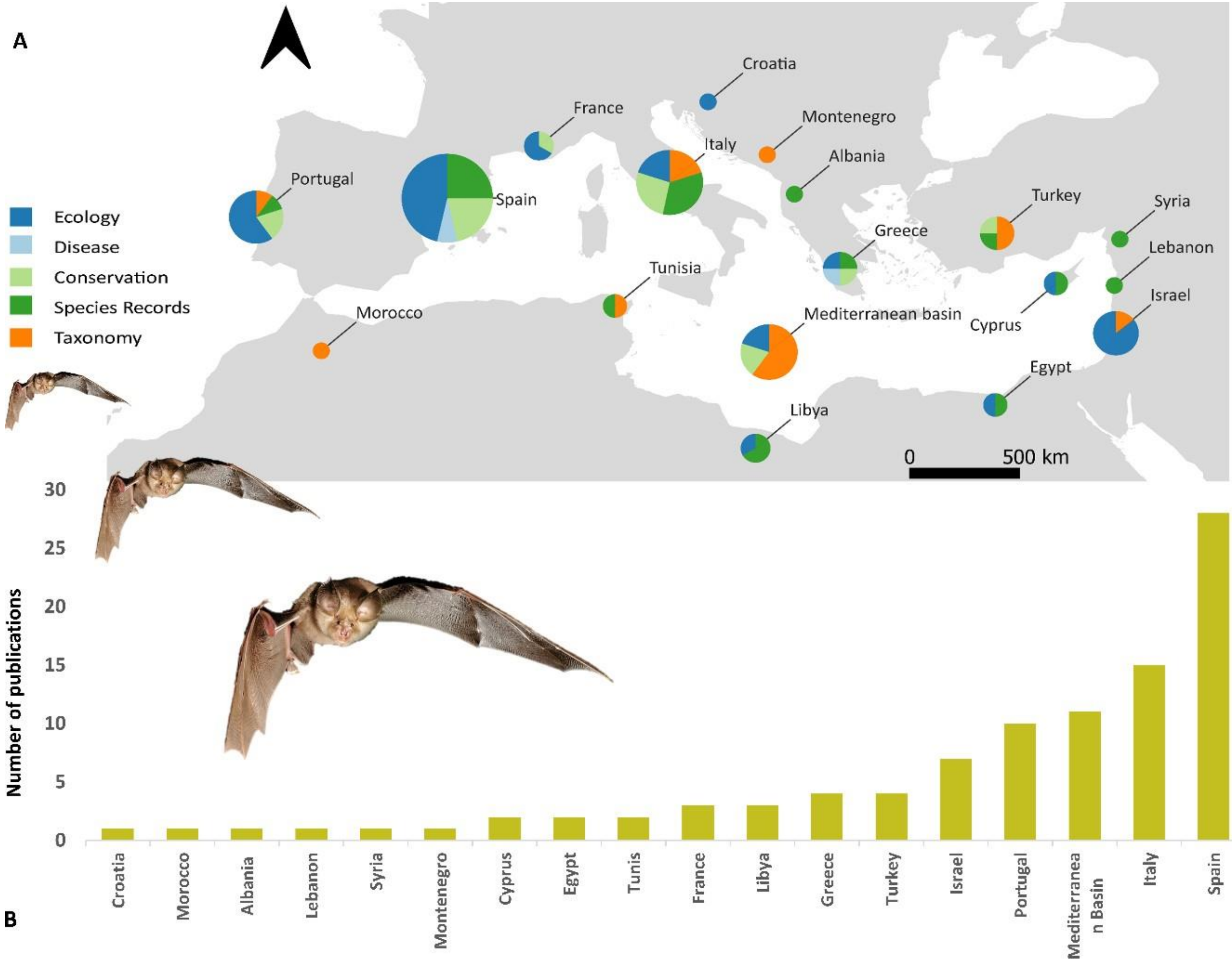


Figure 9. A: Map revealing the geographical distribution of the studies per research thematic topics across the Mediterranean countries (map was created using QGis 3.04 “diagram” function). B: Bar plotshowing the number of published papers by country. The "Mediterranean Basin" designates studies conducted across multiple countries.

Table 2. Distribution of the number of species in the eight families found in the studies and their redlist status based on IUCN's latest classification (IUCN, 2021).

Family	Number of species	Threatened species (% threatened species)	Number of cited times
Vespertilionidae	45	9 (20%)	381
Molossidae	1	0 (0%)	16
Rhinolophidae	6	3 (50%)	67
Rhinopomatidae	2	0 (0%)	9
Pteropodidae	1	1 (100%)	7
Hipposideridae	2	0 (0%)	2
Emaballonuridae	2	0 (0%)	4
Miniopteridae	2	1 (50%)	2
Total	**61**	**13 (21%)**	**488**

Main threats

Nine major threatening factors affecting the Mediterranean bat population were identified throughout the studied papers (**Figure 10**). Yet around 20.6% of the total papers elicited no information on threats. Forest disturbances, such as fires and deforestation, were pointed out as the most common threat factor (18.3%), followed by agricultural practices in particular the overuse of chemical pesticides (14.8%), climate change (11.5%), water resources, such as water availability and water pollution (8%). Data deficiency of bats was identified as a significant threat factor in 6.8% of the papers. Disease and urbanization had a lower impact on bats, accounting for (3.4 %) and (4.6 %), respectively.

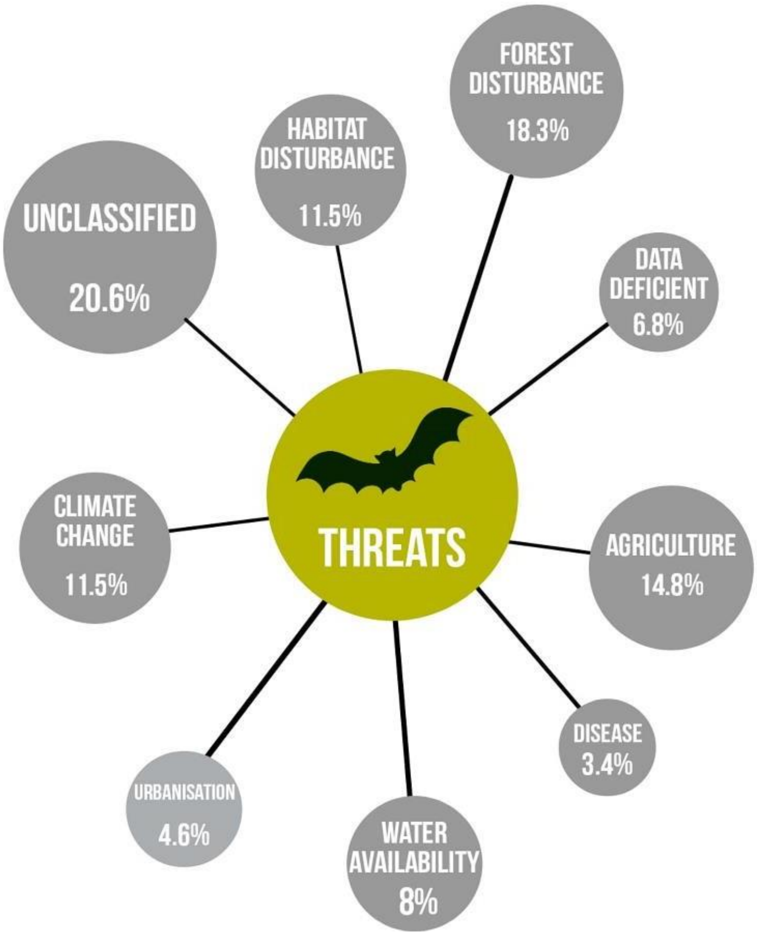


Figure 10. Scheme showing the main threats and factors affecting Mediterranean bats, cited by authors.

Bat activity, species richness, and environmental influence

Bat activity and species richness were assessed in three different types of habitats and four different types of land management. Overall, bat activity averaged 0.87 bat passes/min and ranged between 0.01 (olives groves) and 8.86 passes/min (river). As to bat species richness, species numbers varied between one to 19 species per study. Habitat condition's effect on bat activity and species richness varies significantly among study areas. Bats were found generally more active in riparian areas (ANOVA, $F = 3.67$; $p = 0.022$, $\alpha = 0.05$), than in forest (0.66 ± 0.62 passes/min) (Tukey-Kramer post-hoc-test, p-value= 0.032) and agricultural areas (0.61 ± 0.14 passes/min) (Tukey-Kramer post-hoc-test, p-value= 0.021), where no difference was observed between these two habitats (ANOVA, $F= 0.16$; $p= 0.690$, $\alpha = 0.05$) (Fig. 8). Also, no significant difference in bat activity was detected between managed and unmanaged forest (Tukey-Kramer post-hoc-test, p-value= 0.726) as well as between tradition and organic agricultural (Tukey-Kramer post-hoc-test, p-value= 0.695). Oppositely, species richness did not significantly differ among the different habitat types (riparian, forest, agriculture) (GLM, $\chi2=3.43$, $p = 0.18$). However, unmanaged forests proved to be an important driver for species richness compared to managed forest areas. In agricultural habitats, bat species were found to be more diverse in organic agricultural fields than in conventional agricultural land. In terms of environmental variables, annual precipitation had a negative effect on bat activity ($t= -2.41$, $p=0.019$) and no effect on species richness (GLM, $\chi2=0.55$, $p=0.45$). Contrary, the altitude had no impact on bat activity ($t= 0.44$, $p=0.66$) but influenced positively bat species composition (GLM $\chi2=5.43$, $p=0.01$) (**Figure 11**).

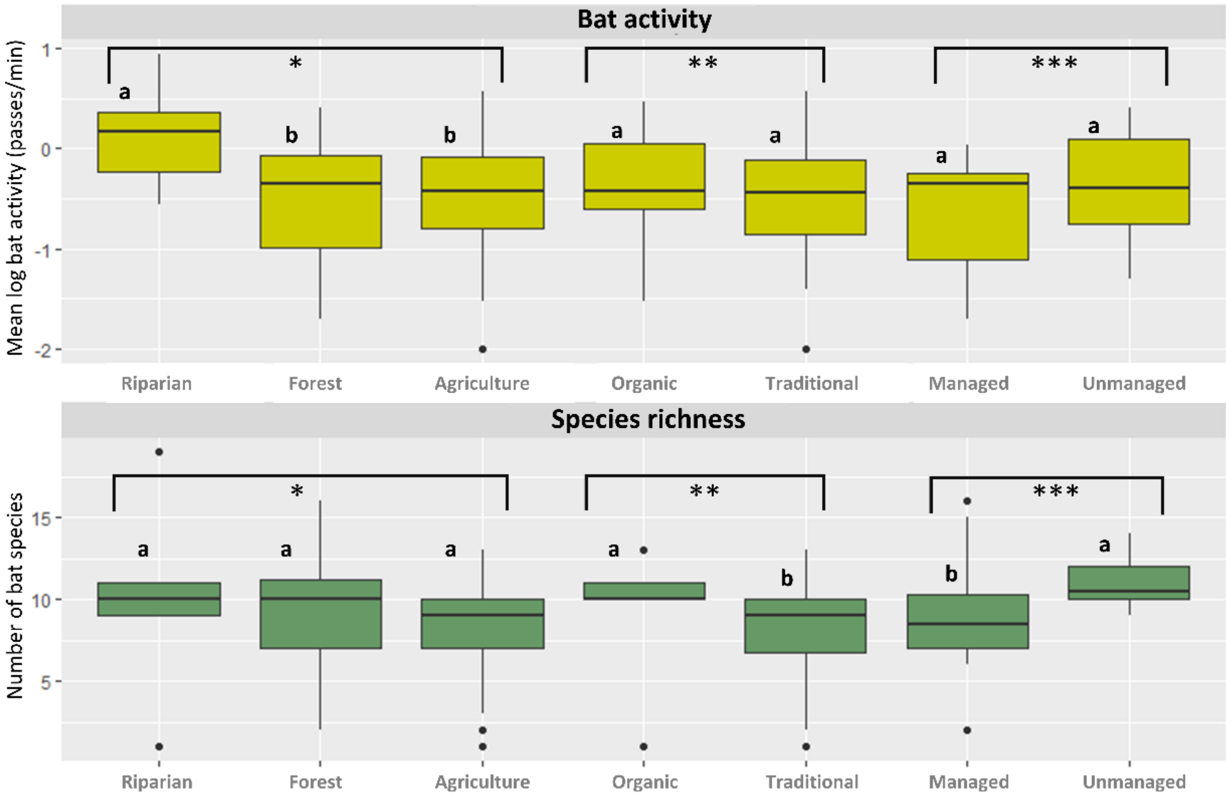


Figure 11. Boxplots representing the variation of mean log bat activity (passes/min) and species richness(number of bat species) in relation to a different type of habitats (*) (agriculture, forest, and riparian areas)and land management: (**) agricultural practices (organic vs traditional) and (***) forest management(managed vs unmanaged) found in the 18 quantitative papers.

2.4 Discussion

Research topic trends

Since the twenty-first century, bat conservation has become a top global priority, and the need to confront and limit all threatening factors has become critical (Frick et al., 2019). In this regard, a significant worldwide increase in the number of studies, culminating in the publication of thousands of papers and reports, paves the way for further research patterns regarding bat taxonomy, status, diversity, ecological role, and diseases. Given that, our review findings confirmed the rise in the number of publications covering a diverse range of research scope related to the Mediterranean bat population over the last years. This increase is highly attributed to a variety of factors, including the advancement of monitoring techniques, and the provision of adequate funds and research grants, as well as the need to enhance our evidence on these species.

Ecology

Despite the expansion in the number of publications in recent years, papers were unevenly distributed across various thematic research topics. Our findings revealed that more than a third of the examined publications were devoted to research related to ecological aspects. The percentage of ecological research papers was significantly greater than that found in related reviews conducted by Tanalgo and Hughes, (2018) in the Philippines and by Feijo et al. (2019) in China and was somehow like the findings of the review conducted by Preble et al. (2020) in Japan. This bias in studying ecology could be due to the need and demand to quantify and evaluate the role of bats in the ecosystem, especially as a large portion of the Mediterranean shift to more sustainable agriculture. In our case, and since most bats are insectivores, research mainly highlighted bats for their role as insect prey predators rather than their pollination or seed-dispersing aspects.

In Spain, Puig-Montserrat et al. (2015) estimated the economic benefits of bats by around 22.5 euro/ha per year. This result was consistent with several studies conducted in various agricultural fields around the world, such as cornfields and rice fields in Thailand (Wanger et al., 2014), vineyards in France (Charbonnier et al., 2021; Froidevaux et al., 2017), enlightening the economic benefits of bats as a pest controller. Bats have also been praised for their ability to improve crop yields in cotton fields (Korine et al., 2019), and olive groves (Davy et al., 2007).
However, several studies have found that bat ecological roles vary across habitats and could be

affected by environmental factors. For instance, Herrera et al. (2015) discovered that bats had a higher ecosystem contribution in organic agricultural areas compared to conventional agricultural sites. Rainho et al. (2010) demonstrated the difference in bat foraging activity between cluttered and uncluttered forests. While Napal et al. (2013) and Rusch et al. (2017) illustrated the influence of deforestation on bat ecosystem services. On the other side, as the presence of a frugivorous bat was particularly intriguing and spatially restricted to certain areas, several studies validated their role across the basin. In Cyprus, Del Vaglio et al. (2011) emphasized *Rousettus aegyptiacus* negative impact on fruit orchards while ignoring their essential role as natural pollinators and seed dispersers. On the other hand, Hadjisterkotis et al. (2006) highlighted the positive contributions of *Rousettus aegyptiacus* through eating only ripe fruits, resulting in a decrease in fungi and a destructive pest population. Additional quantitative data on bat insect preferences in various vegetation habitats, as well as a better understanding of bat insect suppression, pollination, and seed-dispersal roles, are needed.

Bat diseases

Studies focusing on bat diseases and ectoparasites are still considered very scarce across the basin. Only three studies were related to bat diseases. The earliest research discovered the presence of Lyssavirus in the Spanish bat population by Serra-Cobo et al. (2002). After a while, Stekolnikov and Quetglas (2019) reported a new bat parasite known as trombiculids for the first time in Spain. This limited number of studies may be directly tied to public perceptions of bats, which always posed a challenge to bat conservation, especially following the association of bats with the SARS virus in 2002 (Li et al., 2005). However, social disdain and additional persecution of bat populations are likely to increase, particularly following the recent pandemic (Covid-19), for which bats were accused (Macfarlane and Rocha, 2020). Research on disease-relevant bats, on the other hand, is expected to increase to prevent future unforeseen outbreaks (Lu et al., 2021). Given bats' unpopularity in society and their association with zoonotic disease, future research must subtlety consider the negative consequences to avoid further harm, similar to the recent testimonies of planned bat killings carried out by local communities in Peru (Dur Aida, 2020), India (Goyal, 2021), Australia (Lentini et al., 2020), and Indonesia (Tsang, 2020).

Taxonomy and species records

Over the last 15 years, a particular increase in bat species descriptions was distinguished and has outpaced that of other mammalian species by more than 25% (Burgin et al., 2018). This could highlight the importance of taxonomic research, which is currently regarded as a powerful technique for identifying new world species and subspecies (Tsang et al., 2016). On the local level, a paradigm shifts in the taxonomic knowledge of bat species found in the region was noticed especially after the series of taxonomic and checklist reviews across the Mediterranean countries carried out by Benda et al. in the last 20 years (Benda et al., 2010, 2008, 2006, 2003).

As part of taxonomic methodologies, molecular studies have revealed an unexpectedly high diversity of cryptic lineages among bats worldwide, including Europe (Bogdanowicz et al., 2015; Mayer and Helversen, 2001). Our study highlighted the importance of taxonomic research in identifying new cryptic species, which were previously thought to be the same. Several Mediterranean bat species were lately identified including *Pipistrellus sardus*, an endemic species in Sardinia (Mucedda et al., 2002), and *Pipistrellus kolombatovici,* which has also been reported recently by Ancillotto et al. (2019a) in Italy. Also, the Maghrebian Mouse-eared bat, which was known as *Myotis blythii*, was later designated as a separate species called *Myotis punicus*. Moreover, the encouraging findings of Ibáez et al. (2006) in the IberianPeninsula, which confirm the existence of more than 20% of the bat population with cryptic lineage, have opened the door and prompted other researchers to delve deeper into Mediterranean bat species taxonomy. In 2011, Trujillo and Gonzalez discovered the presence of a new species *Pipistrellus maderensis* which is endemic to Madeira, Azores, and the Canary Islands. One year later, Puechmaille et al. (2012) used genetic differences to identify cryptic lineages within the *Myotis nattereri* species complex. Also, Puechmaille et al. (2014) discovered a new species, *Miniopterus maghrebensis sp. nov*, which was previously known as *Miniopterus schreibersii*. Recently Juste et al. (2018) discovered two new *Myotis nattereri* cryptic bat species using mitochondrial and nuclear DNA sequencing. This continued rise of new species discovery can be a hindrance to conservation as it's difficult to determine the status of newly identified species in a short period (Tsang et al., 2016). Therefore, more accurate research is needed to identify and conserve newly discovered species that have been excluded for years and could have been exposed to unanticipated ecological consequences and threats.

Applied methods

A wide range of methods and approaches was employed to monitor bat presence, estimate their population sizes and distribution, understand their foraging, dietary and migratory behavior, and assess their ecological attributions in addition to their interaction with environmental factors. However, when assessed, acoustic monitoring, DNA analysis, radio-tracking, and morphological analysis were found to be more commonly used.

Our results showed dominance in using acoustic monitoring methods with more than a third of studies. Although these methods require considerable time and effort and are considered labor-intensive techniques and could be challenging when analyzing data of passive monitoring, particularly for species with quiet calls. Acoustic monitoring popularity can be attributed to their ability to provide accurate, robust data on bat population and abundance (Russo and Voigt, 2016). These techniques are expected to become widely used as additional affordable bat detectors are becoming available such as AudioMoth (Hill et al., 2019). However, adapted methods are usually strongly related to the research's aim. Although most of the research has been focused on the ecological role and taxonomic aspects of bats, methods such as stable isotopes are less adapted despite their potential in understanding bat feeding strategies (Herrera et al., 2008) and uncovering bat subtle ecological conditions (Cryan et al., 2012). Though less commonly used methods, such as modelling techniques, must be adapted more frequently in future conservation implementations, particularly since they provide a powerful tool for projecting upcoming threats and conservation challenges (Barros et al., 2021; Razgour et al., 2016; Lison and Calvo, 2013, Bilgin et al., 2012; Rebelo et al., 2010). Hence, an unbiased adaptation of methods will elucidate an understandable and unhindered research spectrum on Mediterranean bats.

Geographical research distribution

Our review showed a geographical bias in the research effort. Most studies were conducted in European countries, particularly Spain and Italy, whereas less research was published in Asian and African countries. This spatial bias may be related to the fact that European countries cover the largest area of the basin and have more conserved natural areas and forest areas, which have served as study sites for several studies, such as Goiti et al. (2004) in Spain (Urdaibai Biosphere Reserve) and Lino et al. (2014) in Portugal. Furthermore, the observed geographical bias may be related to the fact that African and Asian nations are perceived to be less environmentally conscious compared to European nations in addition to the financial limitations that may hamper local scientific evolutions. Also, the recommendations set forth by the EU-drafted Convention and the presence of the legislative agreement, the Habitats Directive (Council Directive 92/43/EEC) in 1992, have spurred European nations to undertake additional measures for bat conservation (Voigt et al., 2018).

Additional collaboration with local researchers, particularly in understudied areas, is strongly encouraged and could play an important role in exchanging research expertise and methodology, as well as increasing conservation and informative research evidence to fill the gap in research efforts across different Mediterranean countries.

Species diversity and taxonomic bias

In our review, 61 species were cited in the 97 studied articles. When compared to global data, Mediterranean bat diversity appears to be less diverse than China (135 species; Feijo et al., 2019), Indonesia (221 species; Maryanto et al., 2019), Colombia (187 species; Solari et al., 2013), and Peru (165 species; Pacheco et al., 2009). Although it has a small geographical range, the Mediterranean basin appears to encounter a more diverse bat population than larger areas like North America (49 species; Harvey et al., 2011) and Europe (51 species; Dietz et al., 2009). This could be due to the Mediterranean's role as a transcontinental location, and meeting point of several bioregions, as well as the availability of water resources, particularly on the European side of the basin, which is thought to be critical for bat survival (Dietz et al., 2009).

Taxonomic bias is still an issue in conservation research since it contradicts research recommendations and leads to the marginalization of many species (Tsang et al., 2016). Unfortunately, we discovered a taxonomic bias at two distinct levels in our review. Firstly, and despite accounting for 21% of the total Mediterranean bat population, threatened bats have received far less attention than non-threatened bats. This research inequality may be directly related to the population decline of threatened bats, as well as monitoring challenges since they are classified as forest or cave bats and may be difficult to reach in many cases (Hawkins et al., 2013). Secondly, a taxonomic bias was observed between species and family research efforts. The most cited species were bats belonging to the Vespertilionidae family, which could logically be attributed to the fact that most of the Mediterranean species' population belongs to this family.

Remarkably, some species were only mentioned once, which could raise concerns and could potentially pose additional threats to their presence in the basin. However, more research onless studied and near-threatened species is urgently needed to fill the gaps to address the pervasive lack of data to improve our understanding of these species' population sizes.

Conservation and main threats

Several anthropogenic and environmental threats continue to imperil Mediterranean bats (Voigt et al., 2016). Forest disturbance was identified as the most devastating threat, causing the deterioration of roosting and foraging sites, particularly by removing old and dead trees (Russo et al., 2016) and unsustainable silvicultural practices (Law et al., 2016). Hence, to counteract such threats, many silvicultural strategies were generated, with bat conservation as a main priority (Law et al., 2016). Bats, as predicted, appeared to be extremely sensitive to environmental changes. Climate change was also identified as a serious threat in different studies, affecting bats' ecophysiology, hibernation or the reproduction cycle activity (Ancillotto et al., 2018). Water pollution is a perilous factor that could harm the bat population, especially those who depend on surface water for drinking or foraging (Rainho, 2007). Bats are also threatened by the overuse of chemical pesticides, which not only causes a decline in insect population but also affects bat health by bio-accumulating in their tissues (Puig-Montserrat et al., 2015). Wind turbines have been found to have a significant impact on seasonal bat migratory species, open space species and mating patterns, and a large number of bat fatalities have been recorded in wind farms across the Mediterranean, including in Italy (Ferri et al., 2016) and Spain (Munoz and Frafan, 2020). Threats appear to differ across continents and are dependent on culture, ecosystem, and human factors. In East Asia, for instance, illegal bat hunting for food trading is regarded as one of the major threats (Mildenstein et al., 2016). Heatwaves and wind energy have been identified as the primary threats to bat survival in Australia (Blakey et al., 2018), whereas in the northern hemisphere, the emergence of an infectious fungal disease of bats, white-nose syndrome (WNS), has resulted in millions of bat deaths (Grieneisen et al., 2015). To date, fortunately, these threats appear to have little impact on Mediterranean bats. However, in some parts of the Mediterranean region, some species are still hunted for traditional folk medicinal purposes (Riccucci, 2012). Despite the primary concern and critical need for global bat safeguarding, conservation efforts have stalled over the last 20 years. This conservation effort is undoubtedly influenced by the low number of papers, insufficient legal protection framework, an inability to enforce protection laws, and a failure to implement sustainable agricultural, forestry, and building restoration practices (Frick et al., 2019). Hence and in the face of impending threats, legislativeand practical conservation measures are required, with a strong emphasis on increasing the number of publications addressing particularly threatened species as a priority in any future conservation studies (Browning et al., 2021; Russo and Jones, 2003).

Bat activity, and richness across habitats and land management

Our categorical quantitative comparisons revealed the effect of different habitat types and systems on bat activity and species richness. Although species diversity did not differ, bat activity revealed a consistent difference among the three habitat types. Riparian areas appeared as the preferred habitat for bat foraging. Indeed, this habitat preference could be directly related not only to the direct fact of the presence of water resources but also to the availability of a higher number of insects that usually emerge near water (Fonderflick et al., 2015), particularly during the summer season (Salvarina, 2016). Our findings were consistent with other

Mediterranean studies that highlighted the importance of riparian areas as foraging sites. Higher bat activity was recorded in riparian areas compared to forest and agricultural areas in Italy (Di Salvo et al., 2010; Russo and Jones, 2003), Portugal (Amorim et al., 2018; Mendes et al., 2014; Rebelo and Rainho, 2009; Rainho, 2007), and Spain (Mendes et al., 2014; Puig-Montserrat et al., 2015; Lison and Calvo, 2011). According to (Lisón and Calvo, 2014), water ponds in Mediterranean forests have higher bat activity and diversity than adjacent forest matrix areas. It was also found that permanent water bodies and riparian areas can significantly influence bat species assemblages and activity (Razgour et al., 2011; Rainho, 2007; Russo and Jones, 2003). Similarly, artificial water sources near foraging areas, such as irrigation ponds and dams, were found to have a positive effect on prey abundance (Ancillotto et al., 2019b; Sirami et al., 2013). Nonetheless, Korine et al. (2015) demonstrated that the quality of the water has a critical impact on bat activity and species richness.

Over the last two decades, the expansion of intensive agriculture and the overuse of chemical pesticides in Mediterranean regions has had a significant negative impact on bat richness and activity, as well as insect population (Kolkert et al., 2020). Bats were found more active in organic fields than in conventional fields, most likely due to the negative relationship between agrochemical inputs from one side and insect population and bat activity from the other side (Rodriguez-San Pedro et al., 2018). Our findings strengthened the potential impact of organic agriculture on bat richness and activity and were consistent with previous results (Froidevaux et al., 2017; Toffoli, 2016; Hererra et al., 2015; Davy et al., 2007). Currently, several forest management strategies are adopted across the Mediterranean forests. Based on our statistical approach, bats were found more active in managed forests than in unmanaged areas. For instance, Charbonnier et al. (2014) explained similar findings by linking this preference to an increase in insect population following forest management practices such as clear-cutting and thinning.

However, it is hypothesized that bat response to forest management tends to vary upon species foraging habits. Morris et al. (2010) discovered that forest management has a positive impact on the activity of foraging species in open and edge habitats. Ancillotto et al. (2021) recently concluded that forest management negatively affected clutter-adapted species activity, potentially resulting in the loss of this species foragingsites. More sustainable forest management, particularly those that promote heterogeneity in forest age and structure and retain old trees and snags, is suggested to improve, and conserve Mediterranean bat's habitats and foraging sites (Russo et al., 2016; Lison and Calvo, 2014).

Conclusion

The current review describes the bat status and conservation challenges across the Mediterranean basin, as well as research priorities and gaps. Although the increase in the number of research throughout the year, this number is still insufficient since bats are facing some serious threats. In addition to the limited amount of research devoted to threatened species. Our findings indicate a bias toward certain geographical areas and thematic research, as well as a significant species preference. As predicted, our quantitative findings showed that habitat characteristics, as well as forest and agricultural practices commonly found throughoutthe basin, have a strong influence on bat population activity and species assemblage.

Our review will serve as a basis for future research in the basin. However, several measures are recommended to close current knowledge gaps and implement more effective future conservation: (1) Multinational collaboration among Mediterranean countries in law enforcement and additional research cooperation. (2) To place a greater emphasis on understudied areas, particularly in the African and Asian parts of the Mediterranean. (3) For more effective conservation, more research on threatened and newly recognized species is also required. (4) Carry out additional studies in disease and conservation-related thematic research areas. (5) Improve the legal framework's implementation. (6) Encouragement of sustainable agricultural and forestry practices. (7) Increase the number of research and studies focusing on Mediterranean bats in all thematic and geographical areas in an unbiased manner.

Acknowledgments

We dedicate this work to Prof. Bruno Massa in recognition of all his contributions. This study was supported by a Ph.D. program scholarship from Palermo University. We thank the two anonymous reviewers for their useful comments and suggestions that have improved this manuscript.

Chapter 3

Bats of Sicily: historical evidence, current knowledge, research biases and trends

An adapted version of this chapter has been published in:

Massaad, M., da Silveira Bueno, R., Bentaleb, I., and La Mantia, T. (2023). Bats of Sicily: historical evidence, current knowledge, research biases and trends. Natural History Sciences, 10(2) Click here.

Abstract

Insular bat communities are a preeminent conservation taxon due to their ecological role and intriguing and unique species composition. Sicily is the largest island in the Mediterranean. However, research on Sicilian bats is still scattered, with substantial information being overlooked. Here we present a systematic review of all available bibliographic information from 1810 to 2022, including grey literature, archives, and peer-reviewed publications. The analyses of bibliographic sources permitted us to evaluate the status of research on bats using the Bat Research Efficiency (BRE) and Species-Research Effort Allocation (SREA) metrics. A total of 81 documents were obtained. Since 1955, an average of 1.2 contributions have been issued per year. Over half of the documents are in Italian. The studies are primarily conducted in north-western (40%) and south-eastern (28%) of Sicily, mainly in the provinces of Palermo and Siracusa. Most of the contributions concern "Species records" (61%) and "Ecology" (21%). There were 28 species reported, but a significant bias exists towards *Myotis myotis*, *Miniopterus schreibersii*, *Rhinolophus ferrumequinum*, *Myotis capaccinii*, and *Rhinolophus euryale*. Around 31.5% of the studies focused on threatened species, while 68.5% concentrated on non-threatened species, with an overall preference for cave-dwelling species. Yet, SREA analysis demonstrates a lack of research efforts for all species. We encourage the use of a multidisciplinary approach towards under-studied species while covering geographical gaps and increasing public awareness of the functional role of bats in natural ecosystems.

Keywords: bats, checklist, conservation, Sicily, history.

3.1 Introduction

Islands are renowned for their intriguing patterns of bat species richness and endemism (Conenna et al., 2017; Myres et al., 2002). There are over 1456 species of bats worldwide, with 25% being insular endemics (Burgin et al., 2018). They are considered more vulnerable to irregular conversions and threats (Jones et al., 2009). This entails drastic habitat changes by urbanization, forest degradation, and cave disturbances (Massaad et al., 2022; Frick et al., 2020). Such factors have a downstream effect on insular bat population conservation in general and can influence bat foraging behavior, diet availability, and ecological dynamics across islands (McCreless et al., 2016).

Sicily is the largest island in the Mediterranean and is recognized as a hotspot for biodiversity conservation, given its unique biogeographical patterns, transitional position between Europe and Africa, and its wide range of natural habitats and protected areas (Médail and Quézel, 1999). Currently, 26 insectivorous bat species occur in Sicily (Fichera et al., 2022). They play essential ecosystem roles, particularly as natural suppressors of various pest insects (Puig-Montserrat et al., 2020; Williams-Guillén et al., 2016), and as bioindicators of environmental changes (Russo et al., 2021; Ancillotto et al., 2016). However, the rapid pace of anthropogenic and environmental disturbances, such as agricultural practices, habitat fragmentation, wind turbines, and climate change, are regarded as the primary threat factors affecting Mediterranean bats, including insular bats (Massaad et al., 2022). Hence, an appropriate conservation plan must be implemented immediately to avert irreparable losses.

Research on Sicilian bats is scattered, and limited to brief notes, books, and predominantly Italian-language publications. Hence, relying solely on English or peer-reviewed literature can lead to overlooking important information, thus hampering future research and conservation efforts. In this sense, systematic methods for integrating historical and overlooked datasets can improve the quality of review syntheses and provide detailed insight, allowing us to develop a more comprehensive and less biased overview.

In this context, it is necessary to leverage all existing evidence and available literature to obtain a thorough and representative understanding of the current research status, priorities, and gaps. Hereafter, we intend to present a baseline and future research framework based on historical and current research status. Finally, we aim to provide an updated list of bat species currently represented and reported across the literature.

3.2 Materials and Methods

Literature search and review

A systematic search was developed and implemented between March 10th and April 25th, 2022, following the PRISMA protocol (**Figure 12**) as described by Moher et al. (2015). The systematic research aimed to summarize the evidence and available knowledge about Sicilian bats. Initial datasets were compiled from two types of research sources: (1) primary dataset and (2) secondary dataset documents. The dataset queries were not restricted to any time frame or language barrier.

Primary dataset

Primary datasets were obtained from two different web databases: Scopus (www.scopus.com) and ISI Web of Science (www.webofknowledge.com). The following keywords combination "Bats or Chiroptera or Pipistrelli or Pipistrello" and "Sicily or Sicilia" were used. To cover a wider range of publications, a backward search of the bibliographic sources cited in each publication was performed (i.e. we used citations from one source to find other sources).

Secondary dataset

A bibliographic review of the bats in Sicily was carried out, including historical records, doctoral theses, regional reports, books, monographs, book chapters, webinars, conference papers, conference abstracts, and technical reports. Citations from online repositories such as Bibliografia Teriologica Siciliana. MAMMALIA and Ministero della transizione Ecologica were also checked to collect additional datasets. Furthermore, a search string based on key author names (e.g., Zava, and Catalano) who have made significant contributions to the field of Sicilian bat research was used to include the highest possible number of publications.

Extracted information

After compiling all publications, a primary check was done by sequentially reviewing the titles and abstracts. Then, a detailed full-text evaluation was performed. Studies were excluded if they did not provide any relevant information or data on bat species within the geographical range of the study. When available, the following information was retrieved: author names, year of publication, language (English, Italian and French, and German), geographical location (categorized by province: Agrigento, Caltanissetta, Catania, Enna, Messina, Palermo, Ragusa, Siracusa, and Trapani), publication type (article, abstract, book, online blog), cited species, methodology used (acoustic monitoring, morphology, molecular, observations) and study habitats (forest, caves, laboratory or riparian areas). Eligible articles were then categorized into four main thematic research topics: "Conservation" (conservation status, threats, and anthropogenic pressure, legal protection), "Disease" (Zoonotic disease, viruses, parasites, bacteria), "Ecology" (foraging, roosting, ecosystem role, niche modeling, paleoecology, behavior) and "Species records" (status, distribution, preliminary survey, systematic survey, and new records). We supplemented this literature search with a search of the IUCN Red List database (IUCN, 2022) to identify the trend status for each bat species population (decrease, increase, unknown, and stable), and IUCN Red List threat categories (least concern, near-threatened, data deficient, vulnerable) of the bat species considered. Then, based on the most recent IUCN Red List status assessment, bat species were subdivided into two subgroups. Species classified as data deficient (DD), vulnerable (VU), and endangered (EN) were grouped as "threatened", whereas species classified as least concern (LC) or near threatened (NT) were grouped as "non-threatened" (Massaad et al., 2022). Scientific names of species conform to the names currently used by the IUCN Red List. For example, *Myotis oxygnathus* (Ragonese, 1991) = *Myotis blythii*; *Pipistrellus savii* (Krampitz, 1957) = *Hypsugo savii*; *Vespertilio schreibersii* (Lilford, 1862) = *Miniopterus schreibersii*; *Vespertilio kuhlii* (Lilford, 1862) = *Pipistrellus kuhlii.*

Data analysis

Due to the high heterogeneity of the secondary dataset, data extraction, integration, and analysis proved to be less straightforward. Studies with missing data were excluded from the statistical analysis. The distribution of publications per thematic research topics (Conservation, Ecology, Disease, and Species records), species, language of publications, family, province, and study area was evaluated using Pearson's Chi-square independence test ($\chi 2$). Kendall's Tau-b coefficient was used to identify the presence of any publication trend over the years. The Kruskal Wallis test was performed to check any significant difference in the number of publications over three-decade intervals (1990-2000, 2001-2011, and 2012-2022). All descriptive statistical analyses and plots were generated using R studio packages (R Core Team, 2021).

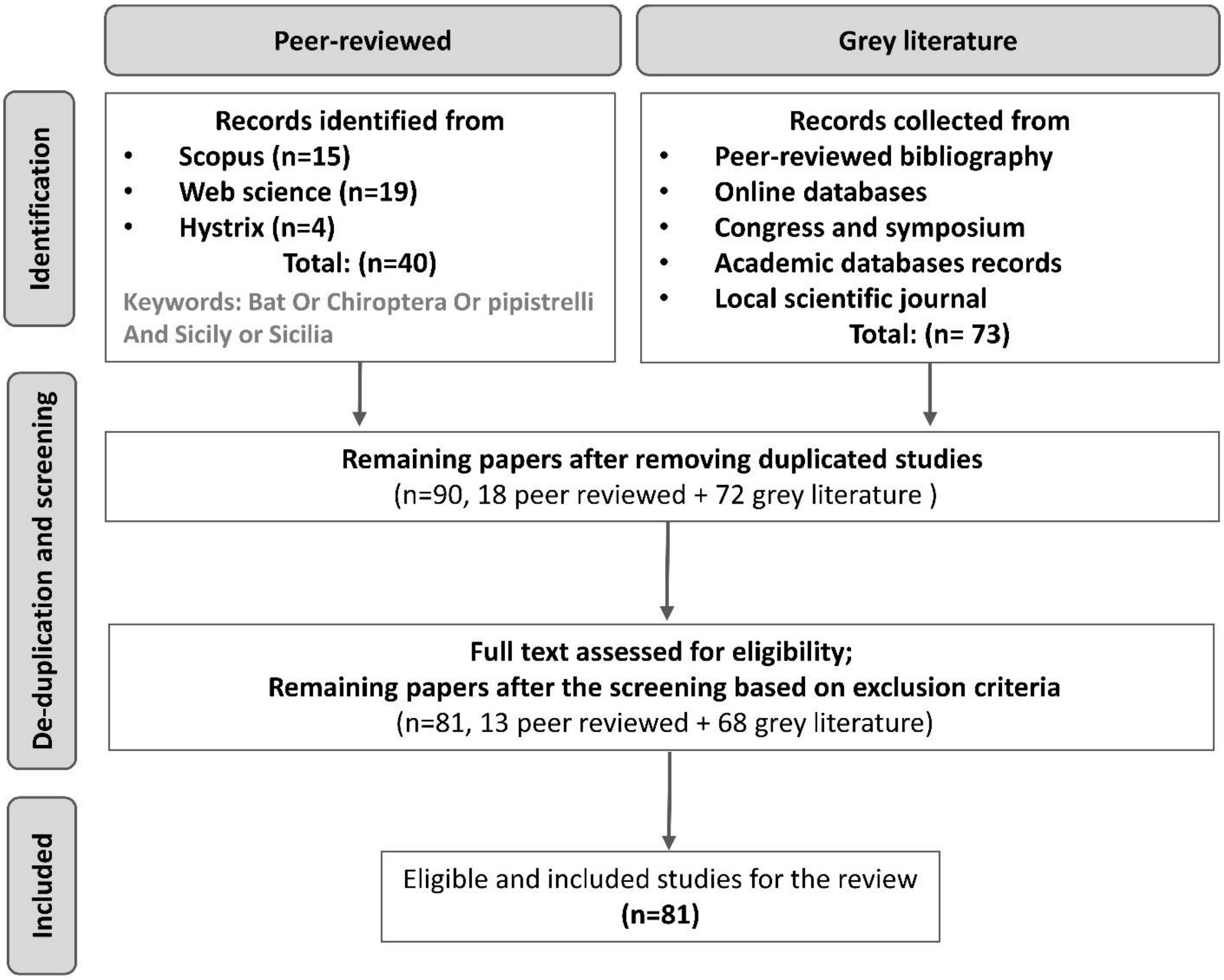


Figure 12. Flow diagram showing the selection steps of eligible articles obtained from primary and secondary datasets.

Bat Research Efficiency (BRE)

The 'Bat Research Efficiency' metric function general idea was adapted from López-Bosch et al. (2021) and then adjusted based on the available parameters. Bat Research Efficiency represents the research efficiency and the accuracy of detection of bat species across a specific geographical area. BRE was calculated using the following parameters: the number of studies conducted in each province (Z), the number of distinct recorded species per province (S), and the area of the province (A).

$BRE=(Z+S)/A$

All the parameters were standardized and ranked as follows:

- (Z): The number of studies per province was ranked between 0 and 1, where 0 indicated the province with 0 related publications and 1 for the province with the highest number of publications.

- (S): The number of species was also ranked between 0 and 1, with 0 indicating no species records and 1 representing the highest number of species recorded per province.
- (A): The province area was ranked between 1 and 2, the number 1 represented the province with the smallest area, and 2 the province with a largest area.

The BRE index ranges between 0 and 1. Regions with a high BRE value exhibit higher research efficiency, higher research publications, and greater taxonomic distinctiveness. Provinces with lower BRE value showed less research effort, a higher research gap, and a shortage of publication and species identifications. Based on the results of BRE, a distribution map of the geographic pattern and research knowledge across Sicilian provinces was created using QGIS 3.0. We excluded publications with undefined geographical localities.

Species-Research Effort Allocation (SREA)

To identify research attention and effort received by each bat species in the period ranging from 1986 to 2022, we utilized an adapted version of the Species-Research Effort Allocation (SREA) metric (Tanalgo and Hughes, 2018). It is worth noting that we selected the period from 1986 onwards, as bat research published has become more static, with little variation in terms of the number of publication items. SREA was calculated using the following metric formula:

$SREA(X) = R/y$

Where (X) denotes the bat species, (R) represents the number of citations per species across the publications, and y was considered as a fixed number of years (36 years, in our case). SREAs with a value of 1.00 refer to species receiving average research effort, SREAs higher than 1.00 refer to species receiving adequate research effort, and SREAs lower than 1.00 represent species receiving insufficient research effort.

3.3 Results

Research trend, source, and language

A total of 81 studies, including 13 primary dataset and 68 secondary dataset documents, were retrieved, and analyzed (**Figure 13**). Since 1955 an average of 1.2 studies have been published per year, with 0.37 for primary dataset and 0.83 for secondary dataset documents. The years 1986 and 2005, with five documents each, stood out with the highest number of contributions. However, the number of contributions has steadily increased since 1994, with an annual average of 1.78 documents (tau-b coefficient= 0.557, $p < 0.05$). Though, no significant difference in the number of documents was observed over the last three decades (1990-2000, 2001-2011, and 2012-2022) (Kruskal Wallis, H-value=2, p=0.391).

The most used language in the contributions was Italian (51%), followed by English with 48% of all publications. Only two documents were written in French, and one in German. However, there was an asymmetrical distribution of languages. Only 10% of primary dataset articles were published in Italian, with the rest in English. The secondary dataset was equally partitioned between documents written in Italian (50%) and English (50%). It is worth noting that the number of documents written in the Italian language has significantly declined in the last 20 years, accounting for only 30% of total contributions, and the rest being written in English.

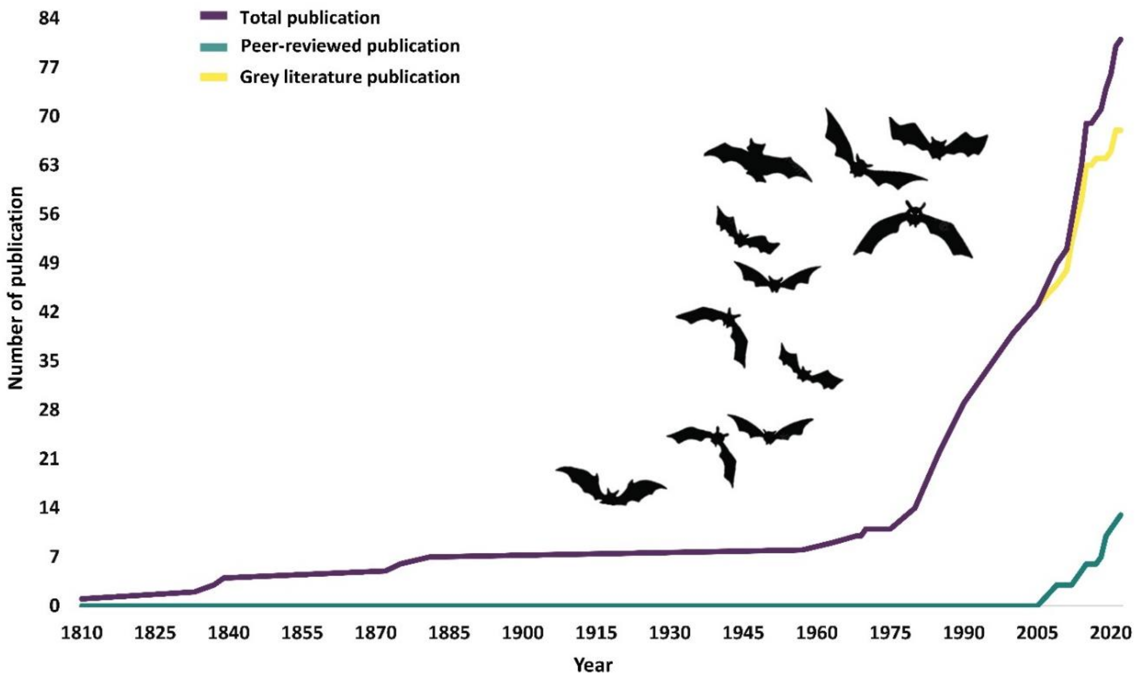


Figure 13. The cumulative number of primary datasets, secondary datasets, and total publications published between 1810 to 2022.

Study areas distribution and BRE

Bat research studies spanned unevenly across the Sicilian provinces. Most of the research occurred in the north-western (40%) and south-eastern (28%) parts of the island. Palermo had the highest number of studies (32%), followed by Siracusa (28%), Catania (12%), and Agrigento (10%). Thus, only six studies focused on Trapani and Messina, respectively. Only two studies addressed bats in Ragusa and Caltanissetta, while only one document was devoted to Enna, and 21 contributions covered more than one region. The higher Bat Research Efficiency (BRE) was accounted for by Siracusa (BRE=0.91), followed by Palermo (BRE=0.71), Catania (BRE=0.58), Trapani (BRE=0.51), Ragusa (BRE=0.48), Messina (BRE=0.41) and Agrigento (BRE=0.36). Caltanissetta and Enna provinces had the lowest BRE value with 0.22 and 0.15, respectively (**Figure 14**). The contributions focused merely on four distinct study areas. There was also an unbalanced distribution of research studies across the study areas. Approximately 54% of the studies focused on caves, 17% on forests, 15% on riparian areas, and only 13% on urban areas (**Figure 15A**).

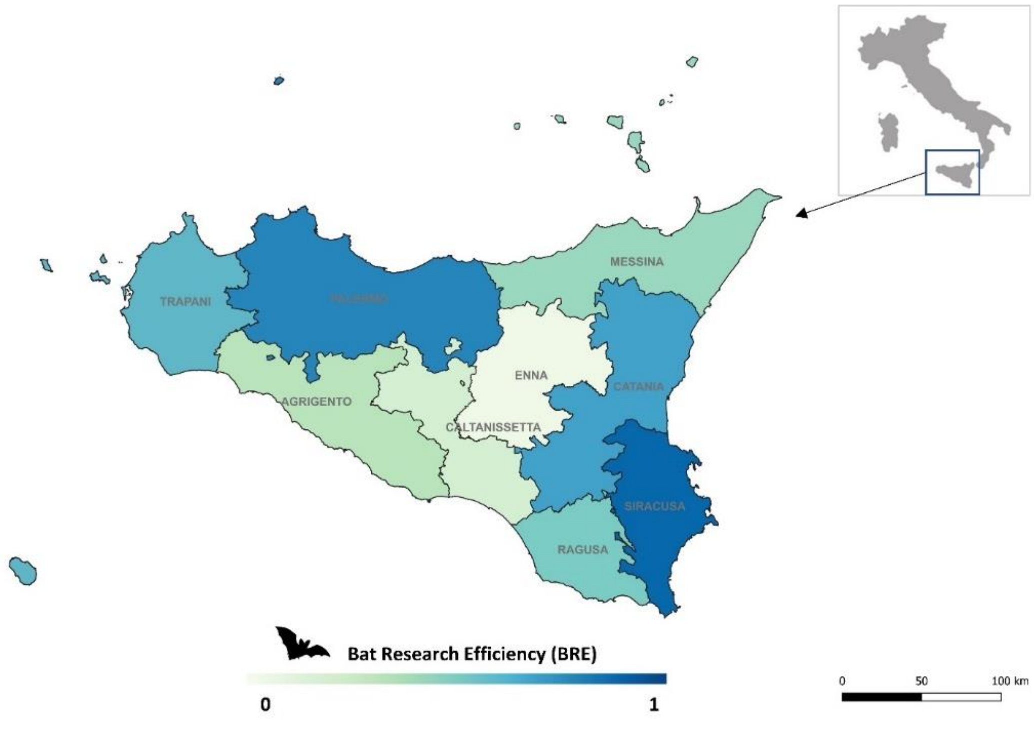


Figure 14. A graphical map displaying the variation in Bat Research Efficiency (BRE) values across nine Sicilian provinces. Darker blue represents the province with a higher BRE, while brighter blue reflects provinces with a lower BRE. The map was created using QGIS 3.04.

Species bias and SREA

A total of 28 bat species belonging to four families were cited across 81 documents (**Table 3**). There was an average of 4.2 bat species cited per document, ranging from 1 to 24 per document. Overall, 21% of the Sicilian bat species were classified as threatened (**Figure 15B**). Around 31.5% of the contributions focused on threatened species, while 68.5% concentrated on non-threatened species, particularly species classified as least concern. Only 15% of the studies focused on a single species. The species of the family Vespertilionidae were the most prevalent among those mentioned (312 times) accounting for 54.5%, followed by Rhinolophidae (30.6%), Miniopteridae (9.9%), and Molossidae (4.8%).

A disproportionate SREA was observed across most of the species, with an overall SREA value below the threshold score of 1.00. The eight most studied species were *Myotis myotis* (n=38 citations, SREA=1.00), *Miniopterus schreibersii* (n=31, SREA=0.82), *Rhinolophus ferrumequinum* (n=26, SREA=0.68), *Myotis capaccinii* (n=21, SREA=0.55), *Rhinolophus euryale* (n=21, SREA=0.55), *Rhinolophus mehelyi* (n=19, SREA=0.5), *Myotis blythii* (n=17, SREA=0.44), and *Pipistrellus kuhlii* (n=16, SREA=0.42). On the other hand, for the following species, attention was scarce, with less than three citations across the 81 contributions: *Nyctalus noctula, Pipistrellus nathusii, Plecotus gaisleri, Rhinolophus blasii, Myotis mystacinus, Myotis punicus, Barbastella barbastellus, Myotis bechsteinii, Pipistrellus pygmaeus.*

Likewise, primary and secondary dataset publications tended to skew towards the same few species (*Miniopterus schreibersii*, *Myotis myotis*, and *Hypsugo savii*). Twenty species were cited in primary dataset publications, whereas eight species were exclusively cited in the secondary dataset (*Rhinolophus blasii*, *Pipistrellus nathusii*, *Myotis emarginatus*, *Myotis bechsteinii*, *Myotis daubentonii*, *Myotis nattereri*, *Nyctalus noctula*, *Barbastella barbastellus*). Only *Plecotus gaisleri* was found in the primary dataset but was not reported in secondary dataset documents.

Thematic focus

Studies revolved mainly around four topics. A significant bias was found across the documents. Studies on bats in Sicily focused mostly on "Species records" (61%), followed by "Ecology" (21%) and "Conservation" (13%), whereas studies on "Disease" were less represented, with only 5% of all publications. However, no statistical difference in research topic preference was observed between English and Italian articles ($\chi 2$ = 3.486, d.f. = 3, p = 0.323). The most frequently covered topics in the English and Italian language documents are 'Species records' and 'Ecology'. Similar findings were observed in primary and secondary dataset publications, with roughly 80% focusing on "Species records" and "Ecology" (**Figure 16**).

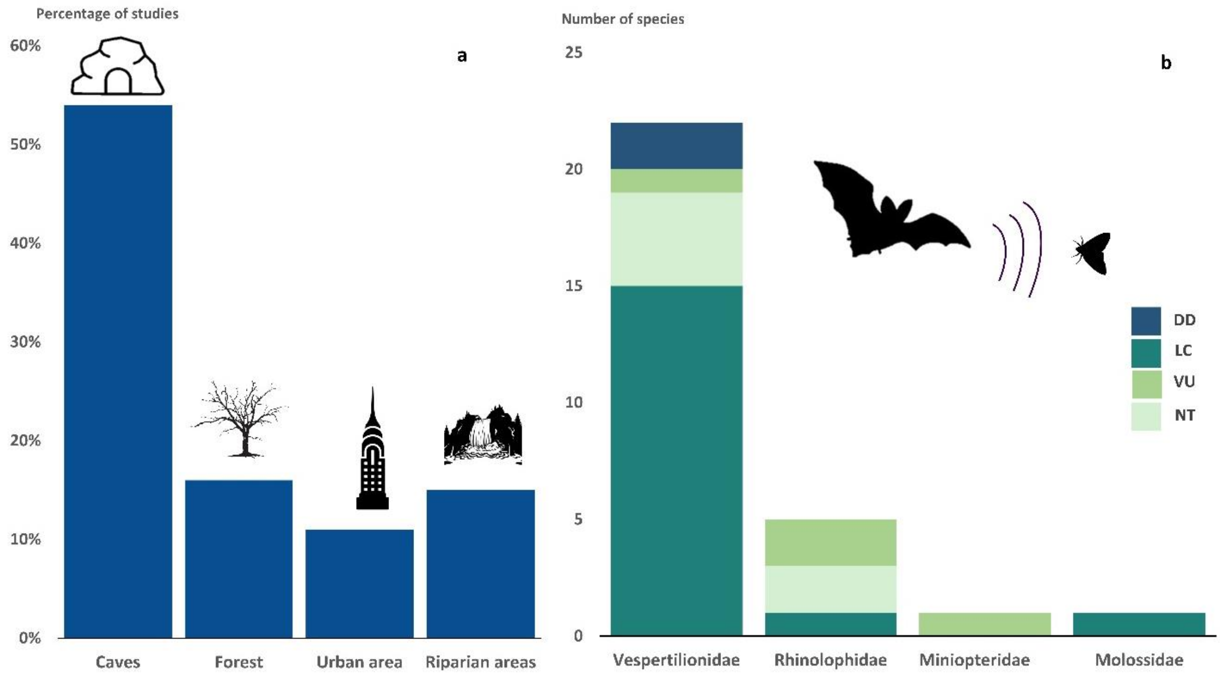


Figure 15. (A): Bar plot showing the distribution of publication across study areas (caves, forest, urban area, riparian areas) (B): Species IUCN status classification across Sicilian bat families. The IUCN conservation status categories are as followed: Least Concern (LC), Vulnerable (VU), Near Threatened (NT), and Data Deficiency (DD).

Adopted methods

Various methods were adopted across the publications. Live capture of individuals and roost observation were the most employed methods, accounting for 26.5% of all publications, respectively. Echolocation (acoustic monitoring) was adopted in 20.4% of the total studies. Approximately, 10% of the contributions used fossil analysis, 2% used isotopic analysis, 6% used genetic analysis, and 4% used chemical analysis and modeling techniques. However, an uneven adjustment of the different methods was found between primary and secondary data (**Figure 16**).

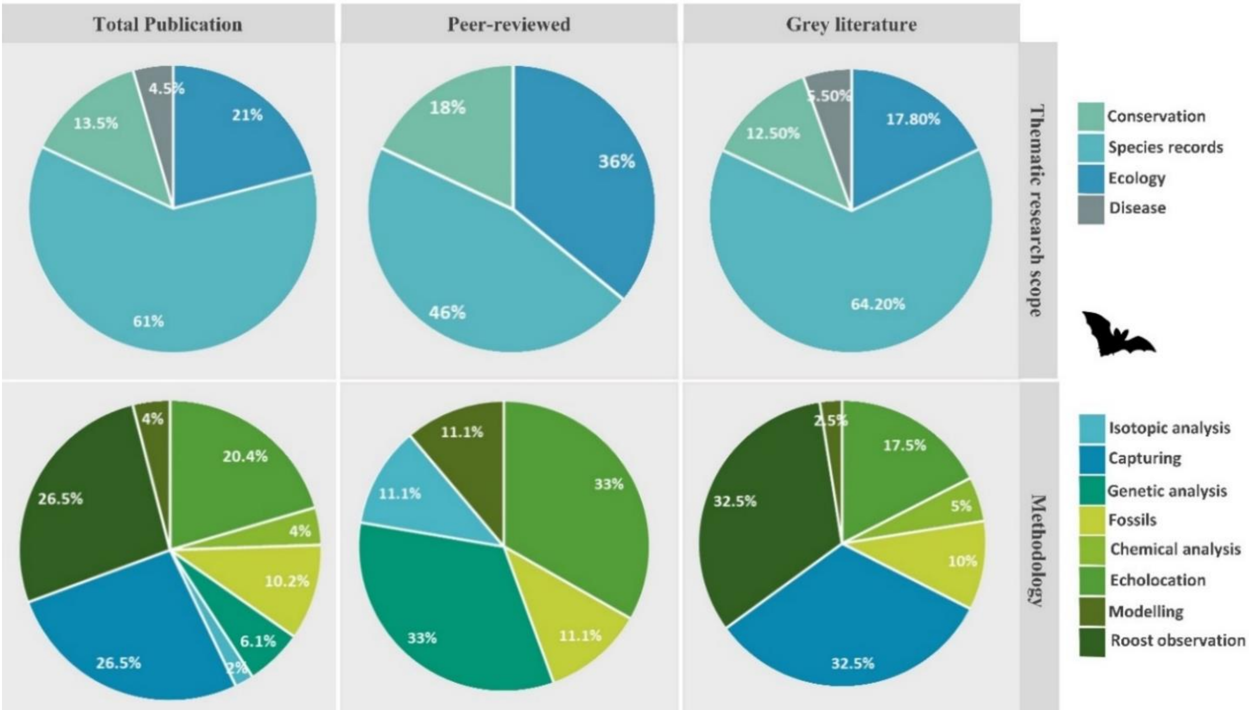


Figure 16. The distribution of the primary dataset, secondary dataset, and total publication according to their thematic research areas and the used methods.

Table 3. List of the 28 Sicilian bat species found across the 81 publications, including the species name, species research effort allocation (SREA), the total number of citations (T.N.C), including those cited across primary dataset publications (PD), Secondary dataset (SD), Population trend and IUCN conservation status. Population trend was indicated by decreasing (↓), unknown (?), and stable (−).

Species name	SREA	T.N.C	PD	SD	Population Trend	IUCN
Rhinolophidae						
Rhinolophus hipposideros	0.5	19	3	16	↓	LC
Rhinolophus euryale	0.55	21	3	18	↓	VU
Rhinolophus ferrumequinum	0.68	26	3	23	↓	NT
Rhinolophus mehelyi	0.5	19	4	15	↓	VU
Rhinolophus blasii	0.02	1	0	1	↓	NT
Vespertilionidae						
Plecotus austriacus	0.21	8	1	7	↓	NT
Plecotus auritus	0.18	7	1	6	—	LC
Plecotus gaisleri	0.04	2	2	0	?	DD
Pipistrellus pygmaeus	0.07	3	1	2	?	LC
Pipistrellus kuhlii	0.42	16	3	13	?	LC
Pipistrellus pipistrellus	0.37	14	5	9	—	LC
Pipistrellus nathusii	0.04	2	0	2	?	LC
Myotis myotis	1	38	4	34	—	LC
Myotis capaccinii	0.55	21	2	19	↓	VU
Myotis emarginatus	0.26	10	0	10	?	LC
Myotis blythii	0.44	17	3	14	↓	LC
Myotis bechsteinii	0.07	3	0	3	↓	NT
Myotis daubentonii	0.10	4	0	4	—	LC
Myotis mystacinus	0.13	5	1	4	?	LC
Myotis nattereri	0.15	6	0	6	—	LC
Myotis punicus	0.07	3	2	1	?	DD
Nyctalus lasiopterus	0.07	3	1	2	↓	NT
Nyctalus noctula	0.02	1	0	1	?	LC
Barbastella barbastellus	0.07	3	0	3	↓	NT
Eptesicus serotinus	0.23	9	3	6	—	LC
Hypsugo savii	0.4	15	6	9	—	LC
Miniopteridae						
Miniopterus schreibersii	0.82	31	4	27	↓	VU
Molossidae						
Tadarida teniotis	0.4	15	3	12	?	LC
Mean	**0.3**	**8.5**	**2**	**6.5**		

3.4 Discussion

Early 19th century bat research

Bats have long captivated the attention of research in Sicily, and this is evident by the presence of historical research records. The first record was reported by Rafinesque (1810), who described and recorded bat species across the island, including an African bat species *Nycteris hispida*. Carlo Bonaparte (1833) provided some earliest evidence of bats on the island and noted the importance of Sicily as a hotspot in Europe, as it holds some unique bat species, especially those belonging to the genus Vespertilio (Sélys-Longchamps, 1839). Subsequently, Luigi Galvani (1837) focused his efforts on the Etna massif and surrounding areas and noted the presence of six species inhabiting the caves of Pantalica-Siracusa and Grotta delle Colombe-Nicolosi. Three decades later, Lord Lilford (1862) reported the presence of seven species in the Siracusa caves. Minà-Palumbo's research (1868) can undoubtedly be described as pioneering, as he was the first to investigate the north-western provinces in 1868, focusing on the bat populations of the Madonie massif and different parts of Palermo and Caltanissetta provinces (Sara, 1999). Doderlein (1881, 1872) reported Sicily Island as the most diverse area in Europe for bats, home to 15 to 16 common bat species including four to five rarely observed species, particularly those found in warmer climates. De Stefani (1895) reaffirmed this in his work on the identification of albinism in museum specimens.

Research temporal trend

Scientific research on bats in Sicily has improved in recent decades, with an increase in the number of both primary and secondary datasets. This rise, specifically for secondary dataset publications, can be attributed to different factors. Firstly, the organization of national or regional conferences and symposia such as; "Italian Conference on Chiroptera" (Mucedda et al., 2015; Fulco et al., 2015a,b; Fulco and Valvo, 2015), Congresso Italiano di Teriologia (Fulco et al., 2016; Mucedda et al., 2015; Russo et al., 2014; Di Salvo et al., 2012a) "Convegno Regionale di Speleologia della Sicilia" (Mucedda et al., 2013; Sperlinga et al., 2013; Caruso 1995). Likewise, the creation of local and national interest groups on bats and the natural sciences in general, such as the Centro Speleologico Etneo (Sperlinga et al., 2013) or the Gruppo Italiano Ricerca Chirotteri (Italian Chiroptera Research Group), has had a significant impact on field research and collaboration between local researchers and academics, constituting a decisive factor in this expansion. Moreover, the availability of grants, access to local open-access journals, and the spread of social media platforms have paved the way for this trend.

Languages and publication sources

The documents examined in the secondary dataset provided a more comprehensive view of taxonomic and historical evidence generally marginalized by publications in the primary dataset. Most of the documents in the secondary dataset were written in Italian as they were mostly published at local conferences and addressed mainly to the local community. This association between native languages and the secondary dataset was previously pointed out by Feijó et al. (2019) for Mandarin Chinese publications. Also, Preble et al. (2021) found a robust link between Japanese written documents and secondary dataset. Although Italian language documents may have an encouraging influence on local conservation, yet they may not be easily retrievable and accessible by non-Italian readers, hampering the dissemination of research results and datasets. To overcome this language barrier and facilitate the dissemination of local research, we endorse the inclusion of English-extended abstracts in future Italian language contributions and vice versa. Also, we recommend the publication of scientific papers in international and local peer-reviewed journals.

Geographical and study area biases

Geographical bias is recognized as a major problem in mammalian research (Guerrero-Casado and Monge-Nájera, 2021). This bias was evident in our review, with more than 70% of the studies carried out in the northwest (Palermo province) and southeast (Catania and Siracusa provinces) of the island. In part, this can be due to the fact that the north-western and south-eastern parts of the island are home to the oldest universities, major academic research institutions, speleological associations, and museums, which tend to concentrate their research efforts in the surrounding areas.

Another reason for this bias can also be elucidated by the presence of distinct ecological patterns and diverse ecosystems, as well as several protected areas that provide unique and facilitated conditions for research. This was also noticeable in the increase in the number of studies carried out in protected areas. One of the earliest confirmations was provided by Kahmann (1957), who discovered the presence of *Barbastella barbastellus*, one of the rarest bat species on the island, in the Ficuzza Natural Reserve. Also, Mannino (1985) carried out extensive research on bat richness throughout the Monte Pellegrino Natural Reserve. Recently, Lo Nigro et al. (2021) detected the presence of 10 bat species identified through ultrasonic monitoring across the same area. Moreover, a few recent studies described the bat populations across the Madonie Natural Reserve (Fulco et al., 2015a; López-Garcia et al., 2013). On the other hand, a substantial amount of bat research was carried out in the Nebrodi Natural Reserve, which encompasses the island's most extensive and diverse forest region on the island (Fulco et al., 2015a; Mucedda et al., 2012; Di Salvo et al., 2012a; Salicini et al., 2011; Agnelli et al., 2008; Vergari et al., 1998; Zava and Violani, 1992; Zava and Lo Valvo, 1991; Zava et al., 1986). To date, a total of 12 bat species have been observed in the Nebrodi mountains, including the recent rediscovery of *Myotis bechsteinii* by Di Salvo et al. (2012b) and *Barbastella barbastellus* by Mucedda et al. (2012).

As for our findings, Siracusa and Catania continue to receive considerable research attention, notably, research focusing on cave-dwelling bats, particularly in the Grotta dei Pipistrelli, Grotta Palombara, Grotta Immacolatella, Grotta di Pantalica, and especially in the Cave of Calafarina, which are famed sites for roost, swarming, mating, maternity, and hibernation for poly-colonies of hundreds of bats (Caruso and Grasso, 1996; Zava and Falzone, 1978; Ragonese 1968). This was in line with the early findings by Ragonese (1967) who highlighted the role of these caves as a home for thousands of bats, *Rhinolophus ferrumequinum*, *Myotis myotis*, and *M. capaccinii*. Similar findings were also underlined in different studies (Caruso and Grasso, 1996; Caruso 1995; Zava et al., 1986; Caruso 1978; Caruso and Costa, 1978). Moreover, Mucedda et al. (2015; 2009) recently discovered the presence of *Rhinolophus mehelyi*, and *Miniopterus schreibersii*, two previously undetected species in these caves.

These caves are now the subject of extensive research and periodic monitoring of different aspects, including paleontology, species abundance, and conservation-related (Audra et al., 2019; Spena et al., 2013; Lanza 2012; Agnelli et al., 2004; Ragonese 1996; Kotsakis and Petronio, 1981). The latest research by Fichera et al. (2021) rigorously described the current bat distribution across 31 different caves located in Mt. Etna and reported the presence of 19 bat species. Overall, all studies confirmed the key role of these caves, particularly as roosts for highly diverse groups of bats (Mucedda et al., 2020; Caruso 1999).

Based on our review, we could pinpoint the current geographical gaps in research. For some provinces, only a limited number of studies could be identified. These include Enna, first investigated by Galvagni in 1837, Castrogiovanni (CIT.), and more recently by Fulco and Lo Valvo, (2015). Similarly, little information has been found on Agrigento province (Haberl 2004; Kramptiz 1957) where studies mainly focused on islands pertaining to its jurisdiction, such as Lampedusa (Lanza 2012; Zava and Catalano, 1983).

The Sicilian archipelago is formed by several smaller islands that cluster around the main island. Earlier evidence suggested that these islands are a promising hotspot for some bat species. However, these areas remain little studied, with most research dating back to the mid-to-late

20th century, except those carried out recently on the island of Pantelleria by Ancillotto et al. (2020) and Fichera et al. (2022). Complementary sampling methods combined with new approaches are therefore needed to extend previous results to all surrounding islands, including the Aeolian Islands (Masseti and Zava, 2021; Zava et al., 1994; Fiore et al., 1992; Kahmann, 1957), Egadi Islands (Masseti and Zava, 2021; Fornasari et al., 1997; Zava and Lo Valvo, 1991; Felten and Storch, 1970) and Pelagie Islands (Fornasari et al., 1997; Zava et al., 1994; Kock, 1989; Zava and Catalano, 1983; Felten and Storch, 1970).

Our results reveal a significant bias in the studied area, with nearly 70% of the studies performed in caves. This disparity may have some implications on Sicilian research evidence particularly for species inhabiting less studied ecosystem and habitats. Hence, there is an urgent need for multifaceted and unbiased monitoring and surveillance programs that should address understudied habitats and provinces including forest areas.

Taxonomic diversity

Taxonomy and species identification remain challenging across Sicilian bat populations. In line with these difficulties, several studies have delved into the occurrence of many previously undistinguished or suspected species. An early review by Agnelli et al. (2008) reported the presence of 20 species across the island. Regardless of being cited by Fornasari et al. (1997), *Myotis bechsteinii*, and *Barbastella barbastellus* were excluded from Agnelli's checklist due to doubts about their occurrence across the island.

Later, Sicilian bats underwent substantial taxonomic research, including the discovery of species new to the Sicilian fauna and the rediscovery of previously recorded ones. In 2015, Fulco and Lo Valvo highlighted the presence of 24 species after adding up the occurrence of *Hypsugo savii*, earlier confirmed by Harbel (2004) and Veith et al. (2011), *Myotis bechsteinii*, rediscovered by Di Salvo et al. (2012b), Soprano pipistrelle (*Pipistrellus pygmaeus*) by Fichera et al. (2012) and *Barbastella barbastellus* rediscovered by Mucedda et al. (2012). Elsewhere, Fulco et al. (2015) reported the first record of *Plecotus auritus* in Sicily at an elevation of 1500 m.a.s.l. Recent research by Ancillotto et al. (2020), supported by Fichera et al. (2022), highlighted the presence of a newly recorded species, Gaisler's long-eared bat (*Plecotus gaisleri*) in Pantelleria, bringing the total number of Sicilian bats to 26.

To our knowledge, two more species were present and mentioned in the literature but are missing from the last species checklist. It includes *Pipistrellus nathusii*, which was reported only twice by Fornasari et al. (1994) and Ragonese (1991), as well as *Rhinolophus blasii* and reported by Ragonese (1991). However, the presence of *Rhinolophus blasii* is highly doubtful, as it has been considered extinct in Italy since the 1960s (Rondinini et al., 2013; Bulgarini et al., 1998). *Pipistrellus nathusii*, on the other hand, was most likely confused with other species of the same genus.

Concerning other adjacent Mediterranean insular bat faunas, the Sicilian bat fauna stands out as the most diverse area with a total of at least 26 confirmed species compared to 21 species of Cyprus (Benda et al., 2018), 22 species of Corsica (Corsica Fauna, 2022), 19 of Minorca (Trujillo et al., 2008), 17 species in Crete (Benda el al., 2008), and at least 21 species of Sardinia (Lecis et al., 2018).

Several factors contribute to Sicily's greater bat species diversity when compared to other adjacent Mediterranean islands. One significant factor is Sicily's geographical location, which serves as a crossroads between Europe and Africa. This unique location enables the convergence of various ecological niches and the mixing of species from different regions (Ancillotto et al., 2020). Furthermore, Sicily's diverse landscape, which includes mountains, a wide range of altitudinal diversity, valleys, forests, and coastal areas, provides ample opportunities for bats to find suitable roosting sites, foraging grounds, and breeding areas, resulting in a greater species diversity.

Among the 28 bat species cited, there was a clear research preference for cave-dwelling species, particularly *Rhinolophus ferrumequinum* and *Miniopterus schreibersii*, which were estimated to account for at least 9000 individuals in some caves (Spena et al., 2013), and *Rhinolophus mehelyi*, which is only known to occur in Sicily and Sardinia (Dondini et al., 2014). Also, *Myotis myotis*, *M. blythii*, *M. capaccinii*, and *R. euryale*, were widely reported and commonly identified through various museum specimens and fossil remains (Spena et al., 2021; Salari et al., 2019; Spena et al., 2017). This bias toward cave species may be due to the ease of access of authorized researchers and local amateur naturalists to the protected caves, which coincides with the various ongoing cave monitoring programs. Today, however, most of the cave bat populations are in decline due to environmental and anthropogenic threats, particularly unregulated cave tourism and the extensive logging of the nearby foraging site (Rondinini et al., 2013). Contrary to conservation priorities, another critical species preference is observed. A large portion of the contributions focused on non-threatened species, while only a few studies focused on threatened species, which account for 21% of all species.

Despite our findings being highly debatable, given the uncertain presence of some cited species, our results show that the island's distinct biogeographical features may provide an important potential habitat for species found in the Mediterranean area, yet to be recorded in Sicily. Among the potentially overlooked bat guilds, forest bats are the most elusive and least studied across the island. It is recommended that future field research and monitoring focus on these species to expand our knowledge of the status of bats throughout the island.

Species research priorities and future metrics

Research priorities have mainly focused on the status of species; however, there is a growing consensus that future research priorities and directions should be based on unbiased and holistic metrics. Through our review, we adapted SREA, normally employed in insular mammal studies as an evidence-based approach in recent years; Japanese bat species (Preble et al., 2021), Philippine bat species (Tanalgo and Hughes, 2018), insular bats (Conenna et al., 2016) and marine mammals (Tiongson et al., 2021). Based on numerical data metrics, all Sicilian bat species except for *Myotis myotis* received insufficient research effort. These findings corroborate our hypothesis that the island's research was largely inadequate and did not meet the effort required for each species. Tanalgo and Hughes, (2018) discovered a common lack of research allocation, where only 13% of Philippine bat species received adequate research attention. Preble et al. (2021) obtained similar results, emphasizing the lack of research effort, particularly for threatened Japanese bat species. On the other hand, we should avoid comparing our regional results with those obtained on a national basis data, as the research effort is necessarily greater in the national context than in the regional one.

Although in general, no species received sufficient research effort, our findings revealed a quasi-match between conservation needs and research attention. SREA was higher in four vulnerable species (*Miniopterus schreibersii*, *Myotis capaccinii*, *Rhinolophus mehelyi*, and *R. euryale*) than in the least-concern and near-threatened species. However, certain species, including the newly discovered *Plecotus gaisleri* and those prone to misidentification, like *Pipistrellus nathusii* and *Rhinolophus blasii*, had lower SREA scores. As a way to improve conservation, the inclusion of additional functional metrics can provide new insights and fill research gaps for understudied and newly discovered species.

Research topic preferences

Another research bias was evident throughout the study. The core studies focused on the thematic area of species records and ecology, while little information was available on bat conservation and diseases. Despite the complexity of studying species records, at least six species have been identified in the last 15 years using mist netting, acoustic monitoring, and molecular techniques. Discovered species were generally reported in short communications (Fulco et al., 2015a; Fichera et al., 2012) or in checklists (Agnelli et al., 2008; Zava et al., 1994). However, methodological advancements such as genetic tools, acoustic monitoring, and interdisciplinary research have enabled the discovery and description of new species. Ancillotto et al. (2020), successfully demonstrated this hypothesis by predicting the geographical presence of *Plecotus gaisleri* by using species distribution model (SDM). Its presence was later confirmed by Fichera et al. (2022) using molecular, and acoustic monitoring methods. Unfortunately, the use of molecular techniques is still limited; even so, their adaptation may improve the scrutiny of the critical and isolated taxonomic complexes and the unique phylogenetic composition found across the island, previously described by Hulva et al. (2007), Salicini et al. (2013), Bogdanowicz et al. (2015) and Juste et al. (2018). Thus, further identification may lead to discoveries of new potential insular lineages and cryptic species distinct from those on the adjacent mainland.

In contrast, fewer studies focused on bats' ecological roles and conservation. As per ecological research, no detailed studies addressed the role of bats to date, and most of the research focused and bat behaviour and their interactions with adjacent habitats. For instance, Di Salvo et al. (2009) described bat habitat preference in a Sicilian rural landscape and compared bat activity and species richness across different habitats. Also, Fulco et al. (2016) published another relevant abstract in which they described the distress calls emitted by *M. myotis* species and the behavioral responses eventually resulting. We believe that the lack of ecological studies is directly related to the lack of funding for long-term studies. Urgent research on bat ecology is required, particularly on their role as insect suppressors, their importance in structuring trophic and spatial ecological networks in agricultural and forest areas (Bueno et al., 2021) as well as how bats respond to spatiotemporal vegetation dynamics (Bueno et al., 2020).

Although all Sicilian bats are legally protected under the Convention on the Conservation of Migratory Species of Wild Animals and EUROBATS, the EC Habitats Directive (92/43/EEC), and other joint environmental accords, few monothematic studies addressed conservation issues and threats faced by Sicilian bats. Corrao et al. (1985) conducted early research that claimed the fatal impact of pesticide residues in a colony of more than 600 bats. To date, bats continue to face many threats, including environmental pollution, such as metal accumulation, as recently highlighted by Ferrante et al. (2018), and extensive forest logging by Rondinini et al. (2013). For better conservation, it is necessary to carry out additional research to identify the main threats jeopardizing bats in Sicily.

In a broad sense, research scarcity was also present in disease-related research and was only studied in a few research conducted across the island. The first publication by Krampitz (1957) described the presence of the protozoan *Trypanosoma vespertilionis* in *Miniopterus schreibersii* bat colonies. Histopathological and microscopic analyses revealed that three bat species inhabiting Grotte dei Pipistrelli, including *M. schreibersii*, resulted positive for pneumonia at the splenic level and showed hyperplasia of the white pulp (Salvaggio et al., 2013). Moreover, Witsenburg et al. (2015) revealed the presence of a haemosporidian parasite in bat colonies in Marzamemi. The use of multiapproach techniques and additional regular monitoring can facilitate and serve as proactive steps to avert any future zoonotic outbreaks, such as those recently witnessed during the COVID-19 pandemic. More research is recommended to fill the lack of evidence in the main research topics, particularly ecology, and conservation, which will certainly improve future conservation plans.

Future perspectives

Research on Sicilian bats has experienced a notable increase in publications over time. However, our review highlights the presence of numerous research gaps and biases in several aspects. To fill these gaps, we propose the following future research perspectives.

We endorse additional research in understudied habitats, particularly in forest and urban areas while maintaining the ongoing monitoring in the caves. More research effort should be done on less studied provinces, Enna and Caltanissetta, in particular.

Regardless of the number of studies reporting on species records and taxonomic analysis, more research is essential since the island may still have undiscovered bat species. Fundamental well-distributed research efforts and prioritization are necessary for responses to the uneven distribution of species research efforts. Several methodological approaches, such as molecular techniques and acoustic monitoring, should be employed to ensure effective species research, protection, and conservation. Furthermore, we recommend additional research on understudied, newly discovered, and threatened species.

Further research is needed to fill the current research gaps in ecology and conservation. Special attention is required to cover bat ecosystem roles in agricultural and forest areas. Because the conservation status is still little understood, we strongly encourage to identify the main threats and assist in setting future conservation measures and guidelines.

At the national level, improvements in research collaboration are also essential, particularly between the amateur naturalist and academic research sectors. As this database is likely to expand in the coming year, future local web-based databases and social media pages about Sicilian bats are strongly encouraged to facilitate research accessibility and promote local research works.

Acknowledgments:

We thank Enrico Bellia and Bruno Massa for their invaluable help in bibliographic research. We thank all the authors for their contributions and research on Sicilian bats. We would also like to thank all the naturalists and people in charge for their efforts in the conservation of bats in Sicily. We express our gratitude to the editor and the two reviewers for their valuable comments and suggestions, which have greatly enhanced the quality of this manuscript.

Supplementary information

SI A. Table of Included primary dataset publications (listed in reverse chronological order).

Primary dataset		
Authors	**Title**	**Year**
Fichera et al.	Pantelleria Island (Sicily, Italy): a biogeographic crossroad for bats between Africa and Europe	2022
Ancillotto et al.	An African bat in Europe, *Plecotus gaisleri*: Biogeographic and ecological insights from molecular taxonomy and Species Distribution Models	2021
Spena et al.	Paleoenvironmental considerations on the latest Pleistocene and Holocene micromammals from the Grotta dei Pipistrelli (Hyblaean Mountains, Sicily, Italy)	2021
Salari et al.	The fossil bat assemblages from the Grotta dei Pipistrelli in Pantalica (southeastern Sicily, Italy): Chronological and palaeoecological implications	2019
Batsleer et al.	Maltese bats show phylogeographic affiliation with North Africa: Implications for conservation	2019
Ferrante et al.	Trace elements bioaccumulation in liver and fur of *Myotis myotis* from two caves of the eastern side of Sicily (Italy): A comparison between a control and a polluted area	2018
Bogdanowicz et al.	Cryptic diversity of Italian bats and the role of the Apennine refugium in the phylogeography of the western Palearctic	2015
Dondini et al.	Rediscovery of Mehely's horseshoe bat (*Rhinolophus mehelyi*) in peninsular Italy	2014
López-García et al.	The small mammals (insectivores, bats, and rodents) from the Holocene archaeological site of Vallone Inferno (Scillato, Lower Imera Valley, northwestern Sicily)	2013
Angelici et al.	A Checklist of The Mammals of Small Italian Islands	2009
Di Salvo et al.	Habitat preferences of bats in a rural area of Sicily determined by acoustic surveys [Preferenze ambientali dei chirotteri in un'area rurale della sicilia]	2009
Hulva et al.	New mitochondrial lineages within the *Pipistrellus pipistrellus* complex from Mediterranean Europe	2007
Zava et al.	Bats of Sicilian islands. II. Ustica	1994

SI B. Table of Included secondary dataset publication (listed in reverse chronological order).

Secondary dataset		
Authors	**Title**	**Year**
Fichera et al.	Attuali conoscenze sulla chirotterofauna dell'etna (Catania, Sicilia)	2021
Lo Nigro et al.	New data about Chiropterfauna of the "Monte Pellegrino" Nature Reserve (Palermo, Italy)	2021
Mucedda et al.	Osservazioni sui pipistrelli della Grotta Palombara e della Grotta di Pantalica	2020
Spena et al.	Pleistocene bats (Mammalia, Chiroptera) from Grotta dei Pipistrelli (southeastern Sicily, Italy). Preliminary report	2017
Mucedda et al.	Studio sui chirotteri troglofili della Grotta di Calafarina (Pachino, SR, Sicilia sudoriental	2015
Fulco and	Geographical distribution of the bat fauna of Sicily: current state of knowledge	2015

Valvo		
Fulco et al.	The bat fauna of four cavities in south-west Sicily: microclimatic analysis and phenology of communities	2015
Fulco et al.	First record of brown long-eared bat *Plecotus auritus* (Chiroptera: Vespertilionidae) for Sicily Island (Italy)	2015
Fulco et al.	Buzzing in case of emergency: distress calls in greater mouse-eared bats	2015
Fulco et al.	Effects of the environment micro-variability on a community of cave bats in western-Sicily	2014
Witsenburg et al.	Presence of *Polychromophilus melanipherus* (Apicomplexa: Haemosporida) in *Miniopterus schreibersii* (Mammalia, Miniopteridae) colonies in Italy	2014
Russo et al.	Modeling interspecific competition and niche displacement in insular bats	2014
Fichera et al.	The first record of *Pipistrellus pygmaeus* (Leach, 1825) (Mammalia Chiroptera) in Sicily (Southern Italy)	2013
Salvaggio et al.	Patologie e cause di mortalità in colonie di chirotteri nella grotta dei pipistrelli (sr)	2013
Spena et al.	I chirotteri della grotta dei pipistrelli (sr): un unicum nella sicilia sud-orientale	2013
López-García et al.	The small mammals from the Holocene archeological sites of Vallone Inferno	2012
Di Salvo et al.	Occurrence of Bechstein's bat *Myotis bechsteinii* (Chiroptera: Vespertilionidae) in Sicily (Natura Rerum)	2012
Di Salvo et al.	New records on Woodland bats in the Nebrodi Regional Park	2012
Mucedda et al.	Record of *Barbastella barbastellus* (Schreber, 1774) (Chiroptera, Vespertilionidae) in Sicily after 56 years (Natura Rerum)	2012
Di Salvo et al.	Attività e distribuzione della chirotterofauna in ecosistemi rurali della sicilia centro-occidentale	2011
NEMO Srl	Piano di Gestione del Sito "Natura 2000"	2010
Mucedda et al.	Status del rinolofo di mehely (*Rhinolophus mehelyi*) (Chiroptera, Rhinolophidae) in Italia	2009
Sarà	Atlante della Biodiversità della Sicilia, Vertebri terrestri	2008
Agnelli et al.	Chirotterofauna della Sicilia	2008
Di Salvo et al.	Preferenze ambientali della comunità di chirotteri di un territorio rurale siciliano	2008
Agnelli et al.	Linee guida per il monitoraggio dei Chirotteri: indicazioni metodologiche per lo studio e la conservazione dei pipistrelli in Italy	2004
Haberl	Small mammals of the "foce del fiume platani" nature reserve (Sicily, Italy) and a record of *Hypsugo savii* (bonaparte, 1837)	2004
Masseti and Zava	Storia Naturale delle Isole Pelagie	2002
Lanza	Francesco Minà Palumbo; Catalogo dei Mammiferi della Sicilia	1999
Sarà	Catalogo dei mammiferi della sicilia	1999
Caruso	La fauna delle grotte dell'etna: descrizione e considerazioni	1999
Fornasari et al.	I chirotteri Italiani	1997
Ragonese and Contoli	Atti del convegno su la Fauna degli iblei tenuto dall' Etna Fauna Siciliana a Noto il 13 e 14 maggio 1995	1996
Caruso	L'attuale stato delle conoscenze sulla fauna delle grotte di Sicilia	1995
Fiore et al.	Chirotteri delle isole circumsiciliane	1991
Ragonese	Chirotteri troglofili degli Iblei	1991
Zava and Lo valvo	Distribuzzione e methodichi di censimento del molosso del cestoni in sicilia	1991

Zava and Lo Valvo	First record of *Suncus etruscus* and notes on the bats of Pantelleria island, Italy	1990
Zava	Atlante iconografico della Fauna vertebrata della Riserva Naurale Orientata dello Zingaro (Sicilia)	1989
Zava, Carrao and Catalano	Chirotteri cavernicoli di Sicilia	1986
Zava	La fauna delle grotte del palermitano	1986
Zava and Catalano	Notes sur la presence de *Myotis nattererri* et *Myotis emarginatus* en Sicile	1986
Mannino, Zava and Catalano	Le grotte della cuspide settentrionale dei Monti di Billiemi (Palermo)	1986
Calandra	A study model—the bat colony of Cefalu Cathedral in Sicily	1986
Corrao et al.	Destructive effects of chlorinated pesticides on a bat colony (Chiroptera)	1985
Mannino	Le grotte di Monte Pellegrino	1985
Zava and Catalano	On the presence of *Tadarida teniotis* in Sicily (Chiroptera)	1984
Zava and Catalano	Premiere decouverte de *Miniopterus schreibersi* dans L'ile de Lampeduse	1983
Kock and Nader	*Tadarida teniotis* (Rafinesque, 1814) in the W-Palaearctic and a lectotype for *Dysopes rupelü* Temminck, 1826(Chiroptera: Molossidae)	1983
Caruso	Il popolamento cavernicolo della Sicilia	1982
Catalano, Zava and Calandra	Recovered of the European Free-tailed bat, *Tadarida teniotis* (Rafinesque, 1814) in Sicily (Italy)	1982
Catalano et Zava	La colonia di Rhinolophus ferrumequinum della Grotta del Pinto, partinico Palermo	1981
Zava and Falzone	Brevi note sui chirotteri del palermitano	1978
Caruso and Costa	Ricerche faunistiche ed ecologiche sulle grotte di Sicilia	1978
Felten and Stroch	Kleinsauger von den italienischen Mittelmeer- Inseln Pantelleria und Lampedusa (Mammalia)	1970
Ragonese	Nel Buio di Calafarina	1968
Sichel and Alicata	Sulla presenza di *Rhinolophus mehelyi* Matschie in sicilia	1963
Kramptiz	Ricerche sugli emoparassiti dei micromammiferei selvatici della sicilia	1957
De Stefani	Nota su l albunismo di un pipistrello e sul melanismo di due rettili	1895
Doderlein	Rvista della fauna sicula dei vertebrei	1881
Lilford	Cruise of the 'Zara,' R. Y. S., in the Mediterranean	1875
Doderlein	Alcune generalità intorno la fauna sicula di vertebrati	1872
Sélys-Longchamps	Études de Micromammalogie. Revue des musaraignes, des rats et des campagnols, suivie d'un index méthodique des mammifères d'Europe	1839
Galvani	Fauna etnea ossia materiali per la compilazione della zoologia dell'etna	1837
Bonaparte	Iconografia della fauna italica per le quattro classi degli animali vertebrati	1833
Rafinesque Schmaltz	Caratteri di alcuni nuovi generi di animali e piante della Sicilia	1810

Chapter 4

Bats in Mediterranean Agroforestry: Exploring activity patterns and spatiotemporal dynamic

An updated version of this chapter is under preparation for submission.

Abstract

Agroforestry habitats are anticipated to foster higher biological diversity and serve as crucial habitats for Mediterranean insectivorous bat species. However, diverse vegetation, landscape features, and climatic factors within each habitat can influence bat composition and activity. Here we explore the response of bat activity and species compositions across different habitats, as well as to vegetation structure, landscape scale, and climatic variables. Through acoustic monitoring across 39 sites covering four main habitats (olives, shrubland, woodland, and grassland) in the protected area of Ficuzza, Sicily, we analyzed habitat-specific, season-specific bat activity, and we examined the nightly activity patterns of the recorded species. We identified 15 bat species and phonic groups from 10,957 calls. Our findings revealed no significant variations in bat activity among the studied habitats. Overall, higher bat activity was associated with areas exhibiting higher Diameter at Breast Height (DBH). A positive correlation was noted between open and narrow bat activity and road proximity, while dung density positively influenced narrow bat activity. Climatic factors, such as elevation and moonlight, had a positive impact on overall bat activity. These results contribute insights into the role of agroforestry systems in supporting bat populations and offer valuable considerations on the mixed response of species towards vegetation, landscape scale, and climatic factors.

Keywords: bat- activity patterns- vegetation structures- Italy- acoustic monitoring

4.1 Introduction

Maintaining and preserving natural habitats is a major challenge for Mediterranean species conservation. Establishing legally designated protected areas, such as National Nature Reserves, National Parks, and Regional Parks, is one of the most widely used strategies for biodiversity conservation (Jackson et al., 2009). Natura 2000 is the European network of protected sites aiming to conserve and protect natural habitats and wildlife species across the European Union areas. This network currently covers about 20% of the terrestrial European area, with 50% designated forest areas. However, certain protected areas are still susceptible to various infringements, particularly in the Mediterranean region (Kerbiriou et al., 2018). The Mediterranean natural habitats and protected areas play a key role as a key ecosystem in conserving and protecting several threatened and endemic species. They harbor rich and unique species composition patterns and interactions, including insectivorous bat species (Lisón et al., 2015; Fady-Welterlen, 2005).

Bats are considered excellent bioindicators owing to their high sensitivity to environmental and climatic changes (Russo et al., 2021; Jones et al., 2009). Mosaic natural habitats, comprising agricultural, forest, and pastoral areas, play an important role in shaping bat composition patterns and abundance. They offer optimal space for bat roosting and foraging needs (Russo et al., 2016; Tillon et al., 2018). On the other hand, habitat landscape features and configurations can model various ecological responses and patterns of bat-habitat interactions. Ongoing habitat loss and environmental challenges continue to have a significant impact on natural areas and species habitats, particularly on insular bats (Massaad et al., 2023; Conenna et al., 2017). Thus, environmental shifts at both the micro and macro levels may affect the ecological response of bat composition, abundance, and activity patterns. Bats are recognized as key ecosystem species for their role in suppressing insect pests, yet the influence of habitat composition on bats remains largely unexplored. Insectivorous bats exhibit high mobility and opportunistic feeding behaviors (McCracken et al., 2012). They adapt their foraging and flying behaviors, as well as habitat preferences depending on their eco-morphological characteristics, including wing morphology, echolocation calls, and body size. Bats are classified into three distinct foraging guilds: open, narrow, and edge space foragers (Denzinger and Schnitzler, 2013).

Various landscape and vegetation structures, such as roost availability, prey abundance and diversity, tree height, density and diameter, clutter, and understory density, are known to diversely influence bat composition and activity. These differences are reflected in bat species' preferences for specific habitats. Bat species may respond differently to landscape compositions and variability. For instance, narrow bat species tend to forage through highly cluttered areas and areas with high tree density and understory cover, owing to their capability to maneuver in closed spaces. On the other hand, open-space species are observed to fly larger distances from their roosts and forage in highly open areas within forest corridors and human-altered landscapes (Denzinger and Schnitzler, 2013). As a result, the connectivity and interaction between bats and habitats can vary significantly among species and their respective foraging guild groups. Understanding these variations is crucial for effective further conservation and management strategies (Froidevaux et al., 2021; Ciechanowski et al., 2007).

Bat activity typically exhibits daily and seasonal pattern variations. On a broader scale, beyond landscape scale and vegetation structure, climatic variables such as temperature, humidity, wind, and moon illumination intensity influence spatiotemporal species composition and activity (Appel et al., 2021; Ciechanowski et al., 2007). Given the ongoing environmental challenges affecting the Mediterranean region, particularly insular bats, it is crucial to better understand the impact of biotic and abiotic factors on bat assemblage. Here, we intend to comprehend the influence of landscape structure across different habitats on bat assemblage and foraging activities, assess the habitat preferences of the species, and finally highlight the impact of environmental factors on bats' nightly activity over the season and across habitats within the Nature Reserve "Bosco della Ficuzza, Rocca Busambra, Bosco del Cappelliere e Gorgo del Drago" in the Sicily islands, Italy.

4.2 Materials and Methods

The study site is on Sicily Island (Italy), within the Nature Reserve "Bosco della Ficuzza, Rocca Busambra, Bosco del Cappelliere e Gorgo del Drago" (hereafter "Ficuzza Natural Reserve"). This area falls within the Special Area of Conservation (code ITA020007) as established by the Habitats Directive 92/43/EEC. It covers more than 7000 ha and hosts a wide range of Mediterranean seminatural and natural vegetation types (Gianguzzi and La Mantia, 2004). This great variability depends on the wide altimetric range (470-1613 m a.s.l.) of the area, as well as on the strong influence of the massif rocky peak of Rocca Busambra, exerting a strong influence on the local microclimate. The average annual precipitation across the entire reserve is 752 mm, with a mean annual temperature of 15.1 °C (Servizio idrografico, 2017). Natural forest vegetation is intermingled with afforestation, shrublands, grasslands, pastures, and agriculture areas mainly covered by olive groves and cereals, thus allowing us to assess the influence of main Mediterranean habitat types on bat spatial activity and species assemblage.

The sampling sites were categorized into four main habitats: olives, shrubland, woodland, and grassland (**Figure 17**). Sampling sites were situated in diverse vegetation types encompassing olive orchards, open grasslands undergoing active shrub encroachment processes (Bueno et al., 2023), shrublands dominated by common hawthorn (*Crataegus monogyna* Jacq.), and other Rosaceae (Bueno et al., 2020), woodlands characterized by the presence of Mediterranean pine plantations with stone pine (*Pinus pinea* L.), cypresses (*Cupressus* spp.), plantations featuring ashes (*Fraxinus* spp.), holm oak forests (*Quercus ilex* L.), and, lastly, old-growth downy oak (*Quercus pubescens* Wild. s.l.) forests typical of late developmental stages (Badalamenti et al., 2017).

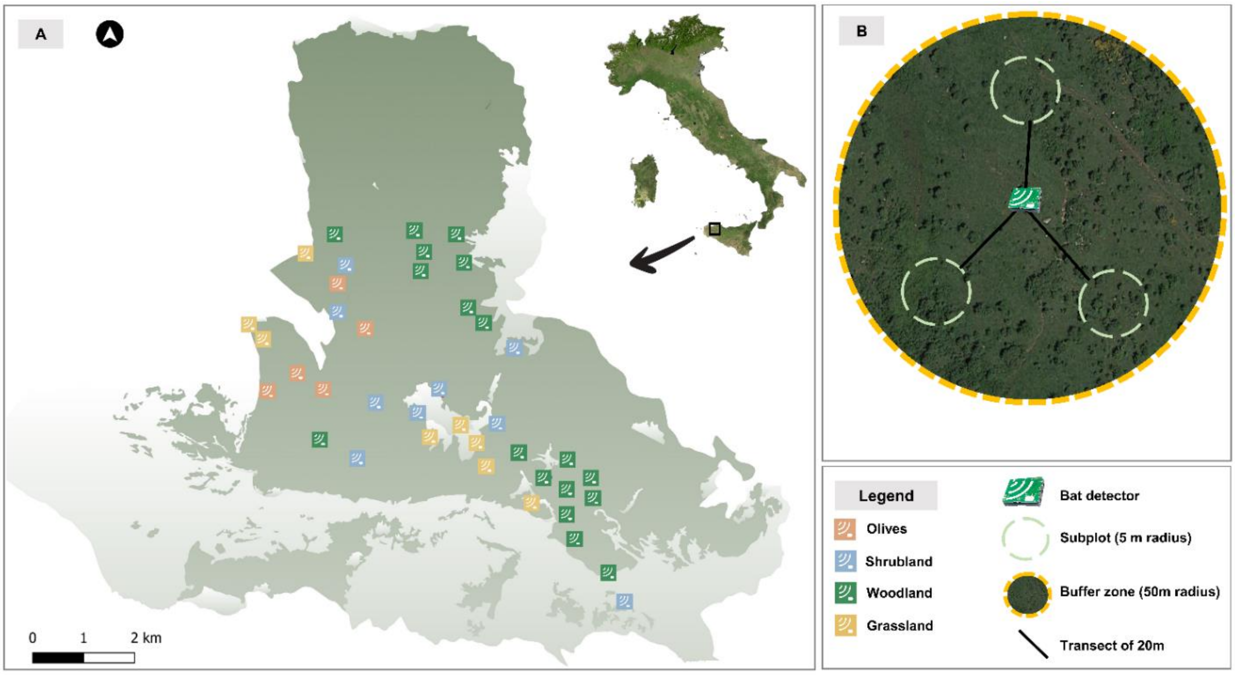


Figure 17. A: a Map of the study area (Ficuzza Natural Reserve) showing the place of sampling sites classified into four main habitats (olives, shrubland, woodland, and grassland). B: a schematic view of the sampling protocol for vegetation structure variables.

Acoustic monitoring

Acoustic monitoring was performed from August until October 2022 and April until October 2023 to record bat occurrence and activity over 262 nights and 39 different sampling sites distributed evenly among the four main habitats: grassland, olive groves, shrubland, and woodland. Bat activity was recorded using AudioMoth 1.2.0 model passive ultrasound recording devices (https://www.openacousticdevices.info). In each sampling round, bat detectors were employed simultaneously across the four habitats. Each bat detector was set at 1.2 m above ground level and angled 45 degrees above the horizontal to avoid echoes in the recordings. Bat detectors were programmed to record 5s for each 1 minute from dusk till dawn, using the following configuration: 384 kHz sampling rate, high-pass filter, and a frequency ranging from 12 to 120 kHz. The number of sampling nights varied between three to six nights. Sampling occurred only on nights with favorable conditions (i.e. with no rain, wind speed < 30 km/h, and temperature at sunset > 10 °C.) To avoid any vandalism incident, a brief note message was included in each bat detector case, outlining the study's purpose, information about instrument usage, and contact information (Ferreira et al., 2022).

Species identification

The classification of calls was performed using automated classifier software Kaleidoscope software v.4.3.2. (Wildlife Acoustics, Maynard, USA). Then we manually verified and reviewed all the calls to minimize misclassified calls, especially for species with low frequencies (circa 10 kHz), often attributed to insects or ambient noises. All calls were then reassigned to species/ or phonic groups based on the descriptive call characteristics of Italian bat calls by Russo and Jones (2002) using the following descriptive call parameters: duration (time between start and end of a pulse), start and end frequencies (frequencies at the start and the end of the pulse), and max peak frequency (frequency of maximum energy of the pulse).

Bat calls were assigned to the species level for most of the calls. In case of ambiguity and difficulties in identifying bat calls at the species level, calls were grouped to the genus level or into phonic groups. This was the case of species belonging to *Myotis* spp. (including all myotis species), *Plecotus* spp. (*P. auritus* and *P. austriacus*), *Nyctalus* spp. (*N. leisleri* and *N. lasiopterus*). In some cases, it was critical to separate *P. pygmaeus* and *M. schreibersii*, therefore we opted to group them as *P. pygmaeus*/*M. schreibersii* group. Whereas unidentified bat calls sequences were assigned as No ID. Then, all bat species/phonic groups were classified into three following functional guild groups according to their foraging guild behavior and functional traits: edge space guild species (*B. barbastellus*, *H. savii*, *M. schreibersii*, *P. kuhlii*, *P. pipistrellus*, *P. pygmaeus*, *P. pygmaeus/M. schreibersii*), narrow space guild species (*Myotis* spp., *Plecotus* spp., *R. euryale, R. hipposideros, R. ferrumequinum*) and open space guild species (*E. serotinus*, *T. teniotis*, *Nyctalus* spp.) (**Table 5**).

During the manual classification process, feeding buzz (a rapid burst of high-frequency echolocation calls emitted during a bat’s foraging), and social calls were noted. Each feeding buzz was considered as a foraging attempt and was considered as foraging activity. In instances, where a 5-second file featured two or more sonotypes, each sonotype was designated as a single bat pass and counted independently. Bat activity was measured by quantifying the total number of bat passes per night. Bat pass was considered for a sequence of at least two recognizable pulses within 5-second WAV files. The foraging activity was calculated as the total number of recorded feeding buzzes per night.

Vegetation structure characteristics

At each sampling site, a buffer zone with a radius of 50 meters was established, centered around the location where the bat detector was deployed. A 50 meters radius was selected based on theoretical considerations matching the bat detector call detection range (Bellamy et al., 2013, Charbonnier et al., 2014). Within the 50 meters buffer zone, three subplots were surveyed. Each subplot had a radius of 5 meters (Area ≈ 79 m2). These subplots were positioned 20 meters from the center plot and aligned along azimuths of 0°, 120°, and 240° to the North direction.

Within each subplot, we assessed tree density (N ha^{-1}), height (H, m), diameter at breast height (DBH, in cm) adopting a minimum DBH threshold of 5 cm, and density of visible tree-related microhabitats (N TreMs ha^{-1}). The international classification of TreMs by Kraus et al. (2016) was considered. Based on DBH and height measured in the field, tree volume (V, m^3 ha^{-1}) of standing trees, both living and dead, was calculated using the double-entry tree volume tables developed within the National Inventory of Forests and Carbon Sinks of Italy (Tabacchi et al., 2011). Based on the DBH value, we also calculated the basal area (BA, m^2 ha^{-1}). Then, we visually estimated woody and herbaceous understory cover in each subplot and classified it as absent (0%), extremely low (0-15), low (15-30), medium-low (30-50), medium-high (50-70%), high (70-90%), or extremely high (90-100%). Other vegetation structure metrics were assessed along three 20 m long transects established from the center plot to the subplots' edge. Along each of the three transects, we assessed dung density by calculating the average count of visible cow dung patties. The canopy cover by the tree layer (%) was determined by calculating the average leaf area cover index within each photo processed by the CanopyCapture® app. A single photo was captured in the middle of each transect with the smartphone positioned at a height of approximately 2 meters above ground level and directed towards the sky. Lying deadwood volume (V, m3 ha^{-1}) was calculated using the following formula by van Wagner (1968):

$$V = (\pi^2 \text{ x } \sum di^2)/8 \text{ x } L$$

Where V (m3 ha^{-1}) is the volume of lying deadwood, d (cm) is the diameter of deadwood at the intersection point with the transect and L (m) is the total length of the transects (in our case 60 m). A minimum diameter of 5 cm and a minimum length of 50 cm was considered for deadwood fragments. Data was collected by the end of the summer season during the month of (September-October). Landscape scale variables, including the distance to the nearest waterways, buildings, unpaved roads, and forest edges, were extracted from QGIS and satellite imagery.

Climatic variables

To understand the influence of climatic variables on bat activity, data including hourly mean temperature (°C), hourly humidity (%), hourly wind velocity (km/h), and length of the day (hour) were obtained from the nearest meteorological station of the SIAS (Servizio Informativo Agrometeorologico Siciliano) regional agency. Additionally, elevation was obtained for each sampling site from (www.elevation.com). To assess the impact of the moon on bat activity and species richness, nightly moon illumination intensity was determined using the open-source software Moonphase 3.4 (**Table 4**;**Figure 18**).

Data analysis

A one-way ANOVA (for normal distributed data) or a Kruskal Wallis (for non-normal) was used to investigate potential variations in bat activity, foraging activity, species richness, and Shannon Diversity Index (H′) across different seasons (autumn, spring, and summer) and habitats (olives, shrubland, woodland, and grassland). In instances where significant differences were detected, a post hoc Tukey or Dunn test was conducted at a significance level of 0.05. The Shannon Diversity Index (H′) of bat species was computed for each sampling site using the "vegan" package, with higher H′ values indicating greater community diversity (Oksanen et al., 2013).

For variable selection, a data-driven approach was adopted to identify the most relevant explanatory variables. Initially, a principal component analysis (PCA) was performed, considering only variables from the first two PCA axes, which accounted for 60% of the variance. The absence of significant correlations between the selected PCA variables was confirmed through a pairwise Spearman correlation analysis. Only variables with correlations below 0.7 were included in the subsequent selection process (**Figure 18**).

To evaluate the impact of vegetation structure, landscape scale, and climatic variables on general bat activity and the activity of each guild group (edge, narrow, and open), a generalized linear mixed-effect model (GLMM) was implemented using the R package "lme4." According to the nature of the response variable (i.e. count data with over-dispersion) we used a negative binomial error distribution with a log link, “lme4” package. The response variables considered were overall bat activity, narrow bat activity, open bat activity, and edge bat activity. Explanatory variables included tree density, mean DBH, herb understory cover, canopy cover, dung density, distance to waterways, distance to unpaved road, distance to anthropogenic structures, and distance to the forest edge. Seasons and habitats were included as random factors to account for the temporal and spatial variability. To assess the impact of climatic factors (temperature, wind, humidity, moon intensity, and elevation), each variable was independently tested through generalized linear model (GLM) for its effect on bat activity of each foraging guild and the most abundant species in each foraging guild group namely: *P. kuhlii* and *P. pipistrellus* (edge), *Myotis* spp. and *Plecotus* spp (narrow) and *Nycatlus* and *Tadarida teniotis* (open). Based on the associations observed, humidity, elevation, and moon illumination intensity were considered as covariates in the models (Froidevaux et al., 2021). We ran all possible combinations of models using the dredge function in "MuMIn" package's (Barton 2017), and the best model candidate for each response variable was selected based on the lowest "AIC" value. Then selected models were evaluated for overdispersion, and zero inflation using the package "DHARMa" (Hartig, 2020). All statistical analyses were conducted using the R statistical software version 4.3.1 (R Development Core Team 2023).

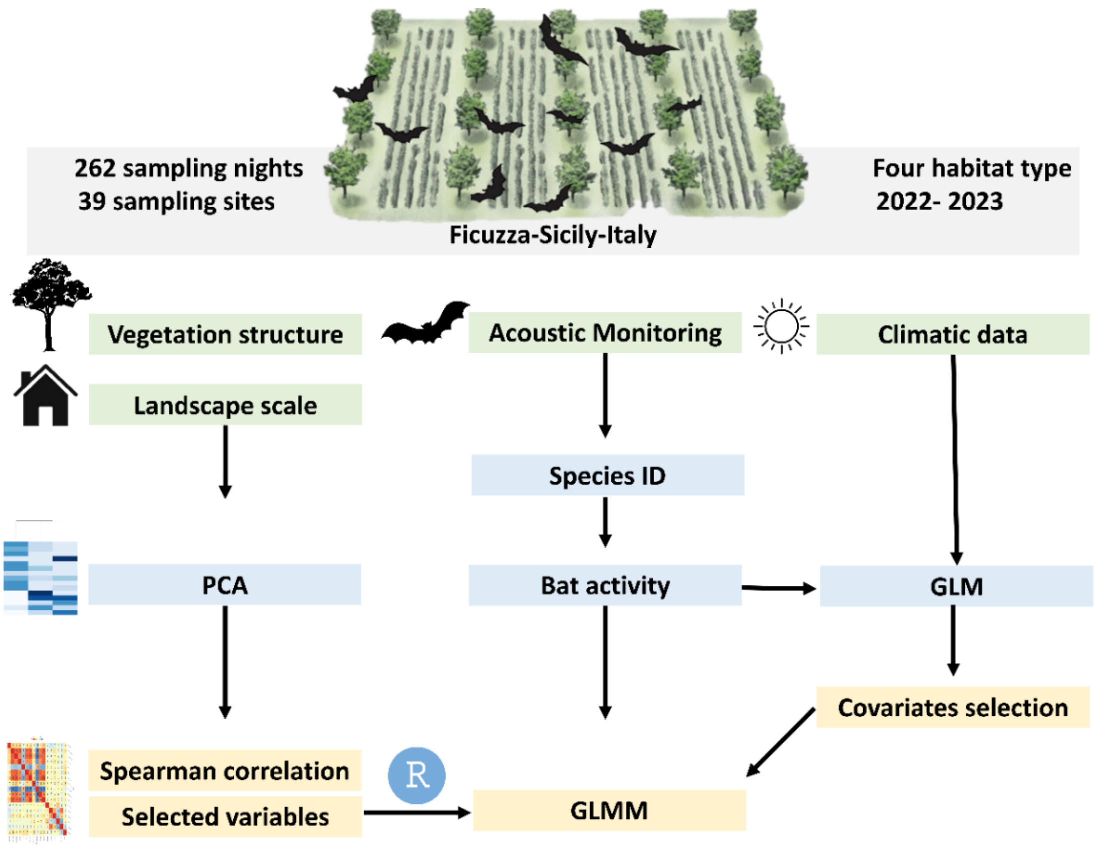


Figure 18. A graphical overview depicting the statistical framework and the sequential procedures employed in conducting data analysis.

Table 4. Vegetation structure, landscape scale and climatic variables with a short description of each variable.

Variable	Unit	Short description
Vegetation structure		
Tree Height	m	Measurement tree height within the subplot using a hypsometer
Mean DBH	cm	DBH was measured at 1.4 meters from the ground for all the available trees inside the three subplots using tree caliper
Tree density	N. trees/ ha	The total number of trees with the three subplots
TreMs	N. TreMs/ha	The total number of tree microhabitats (e.g., canopy deadwood, cavities, and loose bark) encountered across the available trees with the three subplots
Canopy Cover	%	Canopy cover by tree layer (%) was evaluated by the mean average of canopy cover in the middle of each transect
Living tree volume	m^3/ha	The volume of standing living wood trees within the three subplots
Standing Deadwood Vol.	m^3/ha	The volume of standing deadwood trees within the three subplots
Lying Deadwood Vol.	m^3/ha	Lying deadwood volume was calculated using the formula: $V = \pi^2 \sum d^2 8L$ where V (m3) is the volume of lying deadwood, d (cm) is the diameter of deadwood at the intersection with the transect and L (m) is the length of the transect (in our case 60m)
Basal area	m^2/ha	Measure of total live standing wood within the 3 subplots
Woody understory cover	%	Woody understory cover was assessed visually and categorized into the following classes: absent (0%), extremely low (0-15), low (15-30), medium-low (30-50), medium-high (50-70%), high (70-90%), and extremely high (90-100%)
Herbaceous understory cover	%	Herbaceous understory cover was assessed visually and categorized into the following classes: absent (0%), extremely low (0-15), low (15-30), medium-low (30-50), medium-high (50-70%), high (70-90%), and extremely high (90-100%)
Dung density	N. Dung/ha	Dung pellet was counted along the three transect
Landscape scale		
Distance to anthropogenic structure	m	The distance from the sampling point to the closest building structure
Distance to unpaved road	m	The distance from the sampling point to the closest unpaved road
Distance to waterways	m	The distance from the sampling point to the nearest waterways (e.g. water pond, river, or watering trough)
Distance to forest edge	m	The distance from the sampling point to the nearest forest edge
Climatic		
Elevation	m.a.s.l	The elevation of the sampling point from the sea level
Temperature	Celsus	The mean night temperature during the acoustic sampling
Humidity	%	The mean night humidity night calculated along the acoustic sampling
Wind velocity	Km/h	Mean wind velocity night calculated along the acoustic sampling
Moon illumination intensity	%	The percentage of moonlight illumination
Day length	Hour	Day length was considered as the duration of the period of light between sunrise and sunset

4.3 Results

Overall bat and foraging activity

In total, along 262 sampling nights, we recorded 10957 bat passes and 445 feeding buzzes across 39 sampling sites. The average proportional number of bat passes per sampling site was 35.5 bat passes per night (ranging between 0 to 293 bat passes/night). A total of 15 different bat species and phonic groups were identified across the study. The most abundant species was *P. kuhlii* with 4363 bat passes (39.8%), followed by *P. pipistrellus* 3334 (30.4%), and *P. pygmaeus* 1070 (9.8%). The remaining calls correspond to *T. teniotis* with a total of 731 bat passes (6.67%) followed by *H. savii* (5.1%), *M. schreibersii* (2.7%), *Myotis* spp. (2.2%), *Nycatlus* spp. (1.2%), *Plecotus* spp. (0.72%), *E. serotinus* (0.47%), *P. pygmaeus/M. schreibersii* (0.31%), *R. hipposideros* (0.28%) and *B. barbastellus* (0.2%). In turn, two species, *R. euryale* and *R. ferrumequinum* were recorded just 3 and 7 times, respectively (**Table 5**). Overall, a positive correlation was observed between the number of feeding buzz and bat activity (Pearson rank correlation, Rs = 0.72, p > 0.05).

Table 5. List of bat species recorded, along with their foraging guild classifications, total number of bat passes, and feeding buzzes recorded for each species.

Foraging guild	Species/phonic groups	N. bat passes (%)	N. feeding buzzes
Edge space guild			
	Barbastella barbastellus	25 (0.2%)	1
	Hypsugo savii	558 (5.1%)	11
	Miniopterus schreibersii	298 (2.7%)	23
	Pipistrellus kuhlii	4363 (39.8%)	115
	Pipistrellus pipistrellus	3334 (30.4%)	192
	Pipistrellus pygmaeus	1070 (9.8%)	69
	P. pygmaeus/ M. schreibersii	35 (0.31%)	0
Narrow space guild			
	Myotis spp.	239 (2.2%)	16
	Plecotus spp.	79 (0.72%)	2
	Rhinolophus euryale	3 (0.02%)	0
	Rhinolophus hipposideros	31 (0.28%)	1
	Rhinolophus ferrumequinum	7 (0.06%)	0
Open space guild			
	Eptesicus serotinus	52 (0.47%)	1
	Nyctalus spp.	132 (1.2%)	6
	Tadarida teniotis	731 (6.67%)	7
Total		**10957**	**445**

Bat activity and species richness across habitats and seasons

Both in the global and the species guild comparisons, bat activity, richness, and Shannon diversity did not differ significantly between the four habitat types (ANOVA and Kruskal Wallis $p > 0.05$; (**Figure 19**; **Figure 21**; **Figure 21**). There were significant differences between overall and edge bat activity across seasons, with post-hoc Dunn tests indicating a significantly higher activity in summer than in autumn for both guild species groups. Species richness in turn differed among seasons (ANOVA $F = 10.07$, $p < 0.001$), with the post hoc Tukey test indicating a higher number of species in summer than in autumn and spring ($p < 0.001$). As to foraging activity, no statistically significant differences were observed among the various seasons and habitats (**Figure 20**).

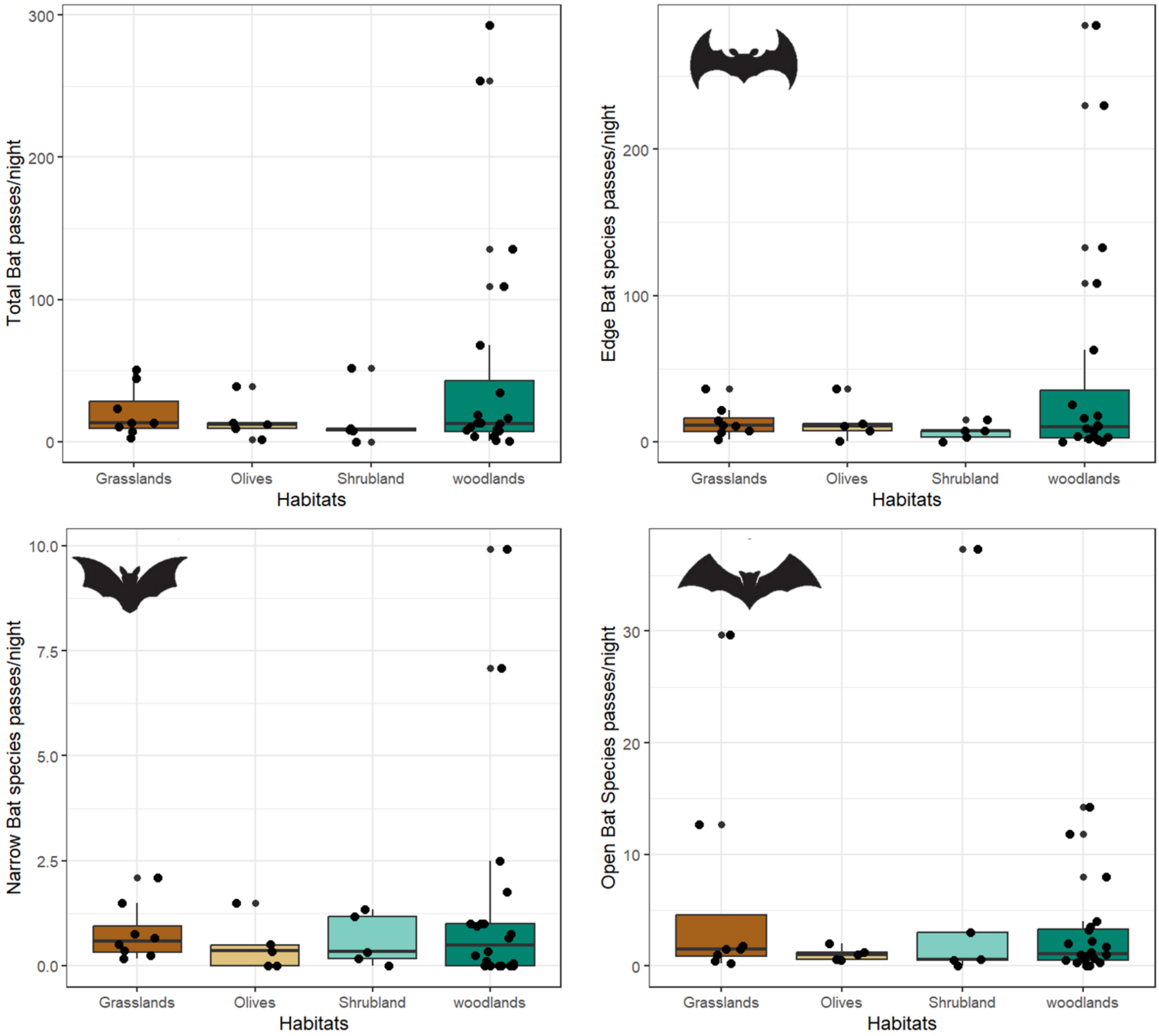


Figure 19. Bat activity in the four habitats for all bat species and three distinct foraging guilds.

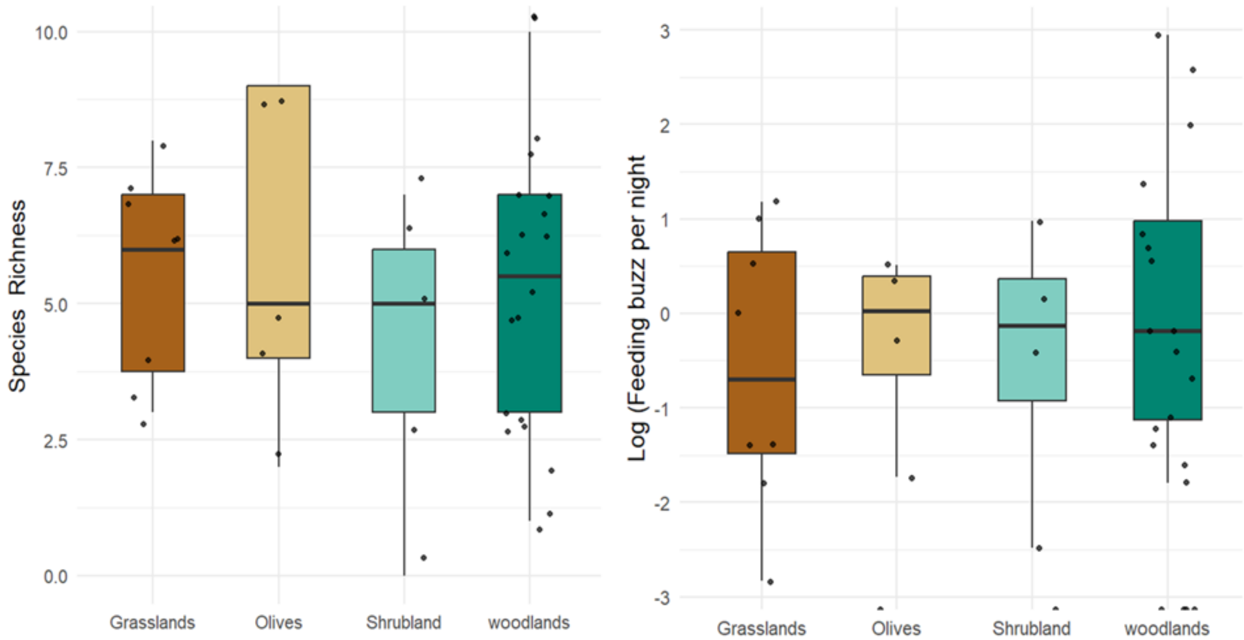


Figure 20. The variation in bat species richness (left) and foraging activity was measured as log feeding buzzes per night (right).

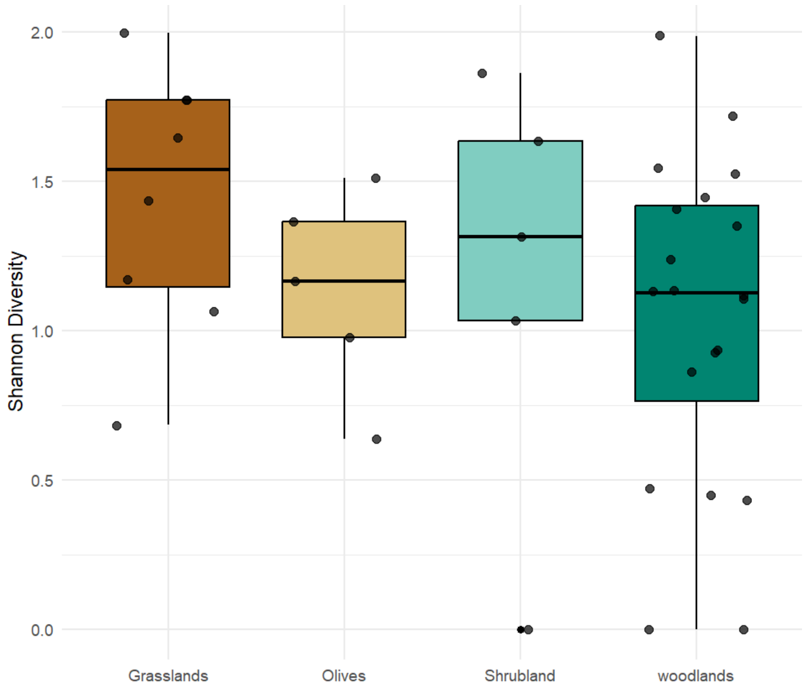


Figure 21. Shannon diversity index of bat species compositions observed among the four studied habitats.

Bats hourly activity

Our findings revealed two distinctive peaks in bat activity. The initial peak occurred shortly after sunset and concentrated within the first three hours, and a second peak was observed a few hours before dawn. Species such as *B. barabstellus*, *E. seronitus*, *H. savii*, and *R. ferrumequinum* exhibited a single peak of activity, with most of their active periods concentrated in the first peak. Other species such as *Myotis* spp., *M. schreibersii*, *P. pygmaeus*, *P. kuhlii*, and *T. teniotis*, displayed two distinct activity peaks, post-dusk and pre-dawn, respectively. As for *Nyctalus* spp., *P. pipistrellus*, and *P. pipistrellus/M. schreibersii* their activity was concentrated over three activity peaks (**Figure 22**).

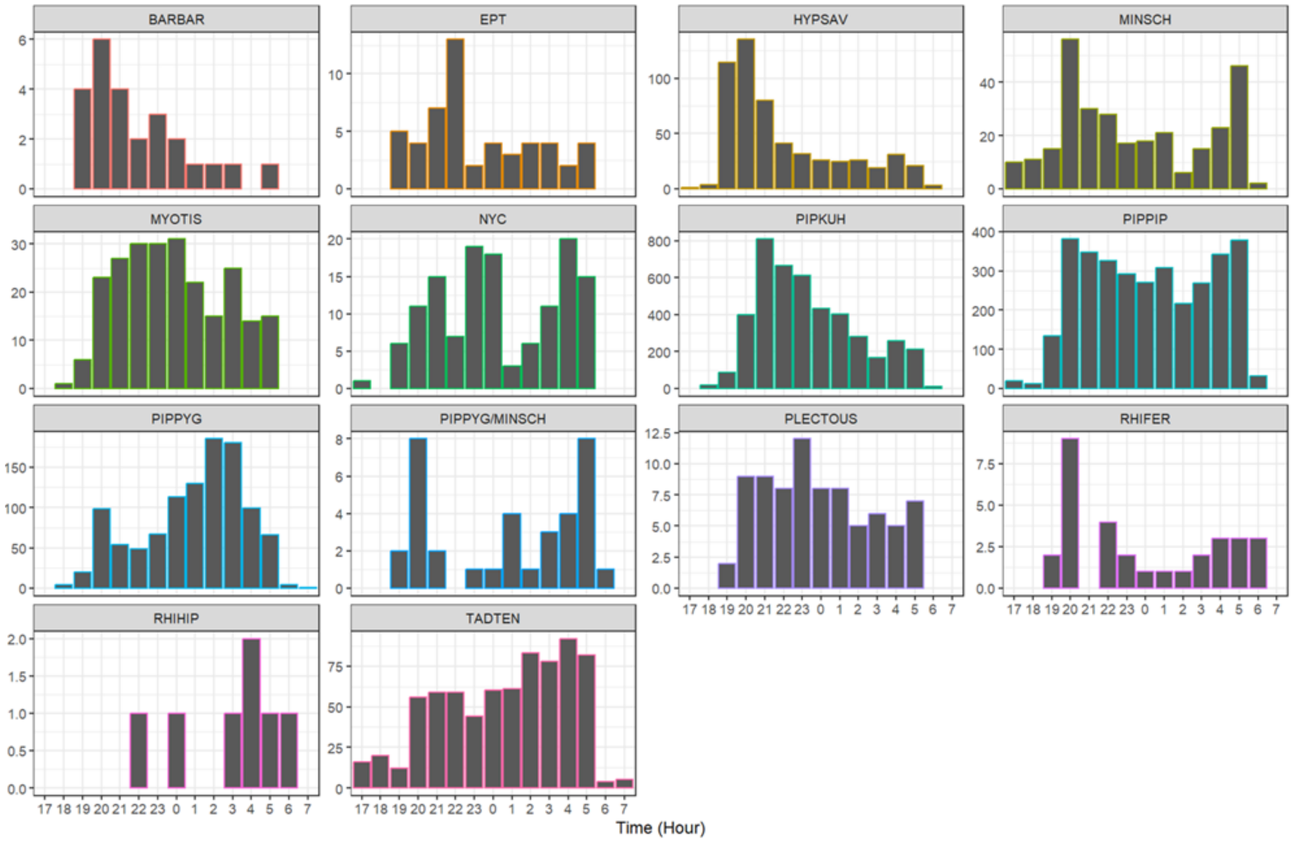


Figure 22. Nightly activity patterns of various bat species, including *B. barbastellus* (BARBAR), E. *serotinus* (EPT), *H. savii* (HYPSAV), *M. schreibersii* (MINSCH), *Myotis* spp. (MYOTIS), *Nyctalus* spp. (NYC), *P. kuhlii* (PIPKUH), *P. pipistrellus* (PIPPIP), *P. pygmaeus* (PIPPYG), *P. pygmaeus/M. schreibersii* (PIPPYG/MINSCH), *Plecotus spp.* (PLECOTUS), *R. ferrumequinum* (RHIFER), and *R. hipposideros* (RHIHIP). The x-axis represents the night sampling hour from dusk to dawn, and the y axis the number of bat passes.

Principal component analysis and variables collinearity

The first two components explained around 60% of the total variation of the variables. The first component PC1 consisted of 44% of the total variation. It included mostly variables associated with vegetation structure including mean DBH, tree density, herbaceous understory cover, tree height, TerMs, basal area, canopy cover, and living volume (**Figure 23**). The second component PC2 represented 16% of the total variation and was highly associated with landscape scale variables including distance to the road, anthropogenic structures, and woody understory cover. Based on Spearman correlation (**SI C**), variables such as tree density, tree height and TerMs, canopy cover, and living volume were highly correlated with DBH ($r>0.7$), on the other hand, no significant correlation was detected among landscape scale variables. Separately, no significant correlation was found between climatic variables, except for temperature and day length ($r=0.76$).

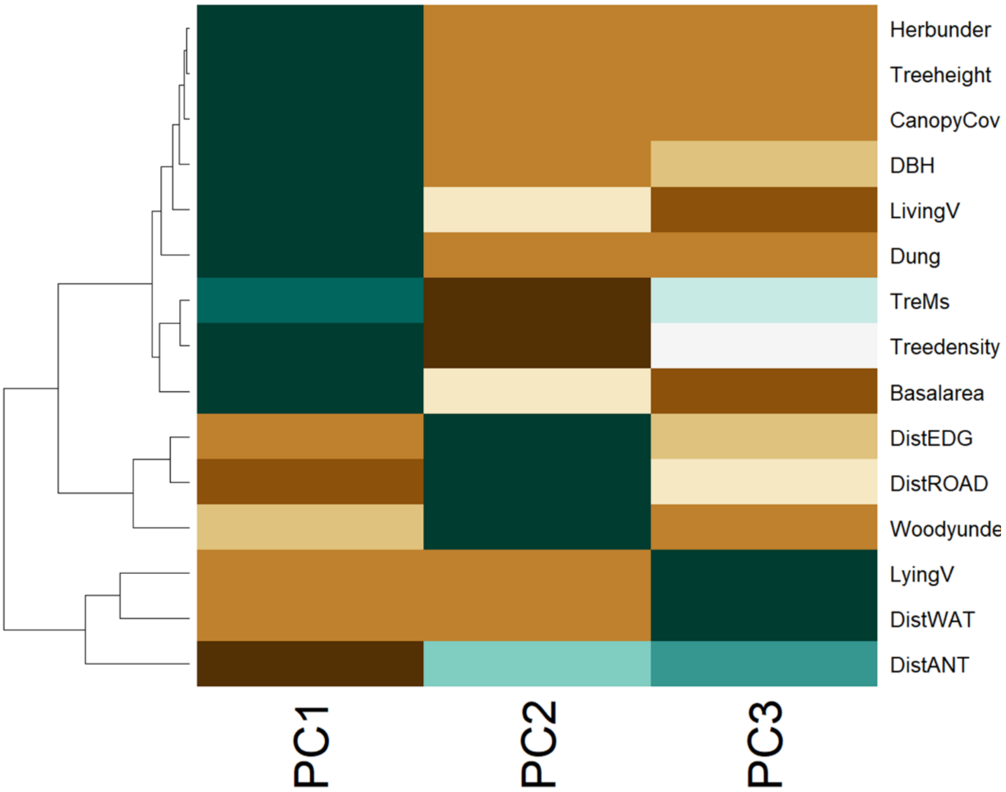


Figure 23. PCA ordination biplot of 15 vegetation structure and landscape scale variables with their respective contribution on the first 3 axes (PC1, PC2 and PC3).

Climatic factor influence

Overall, few significant associations were identified between overall bat activity and climatic variables. Faster wind velocity was negatively correlated with the activity of open species, whereas moon illumination intensity had a positive impact on open species activity (Z= 2.66; p<0.01). On the species level, *T. teniotis* was significantly negatively associated with higher temperatures (Z= -1.834; p<0.046), faster wind (Z=-2.049; p<0.049), and increased humidity, whereas higher moon illumination was positively associated with *T. teniotis* activity (Z= 2.903; p<0.007). *Plecotus* species activity exhibited a negative correlation with higher humidity (Z= -1.765; p<0.05). *P. pipistrellus* activity demonstrated a strong positive relationship with higher elevation and humidity (**Figure 24**).

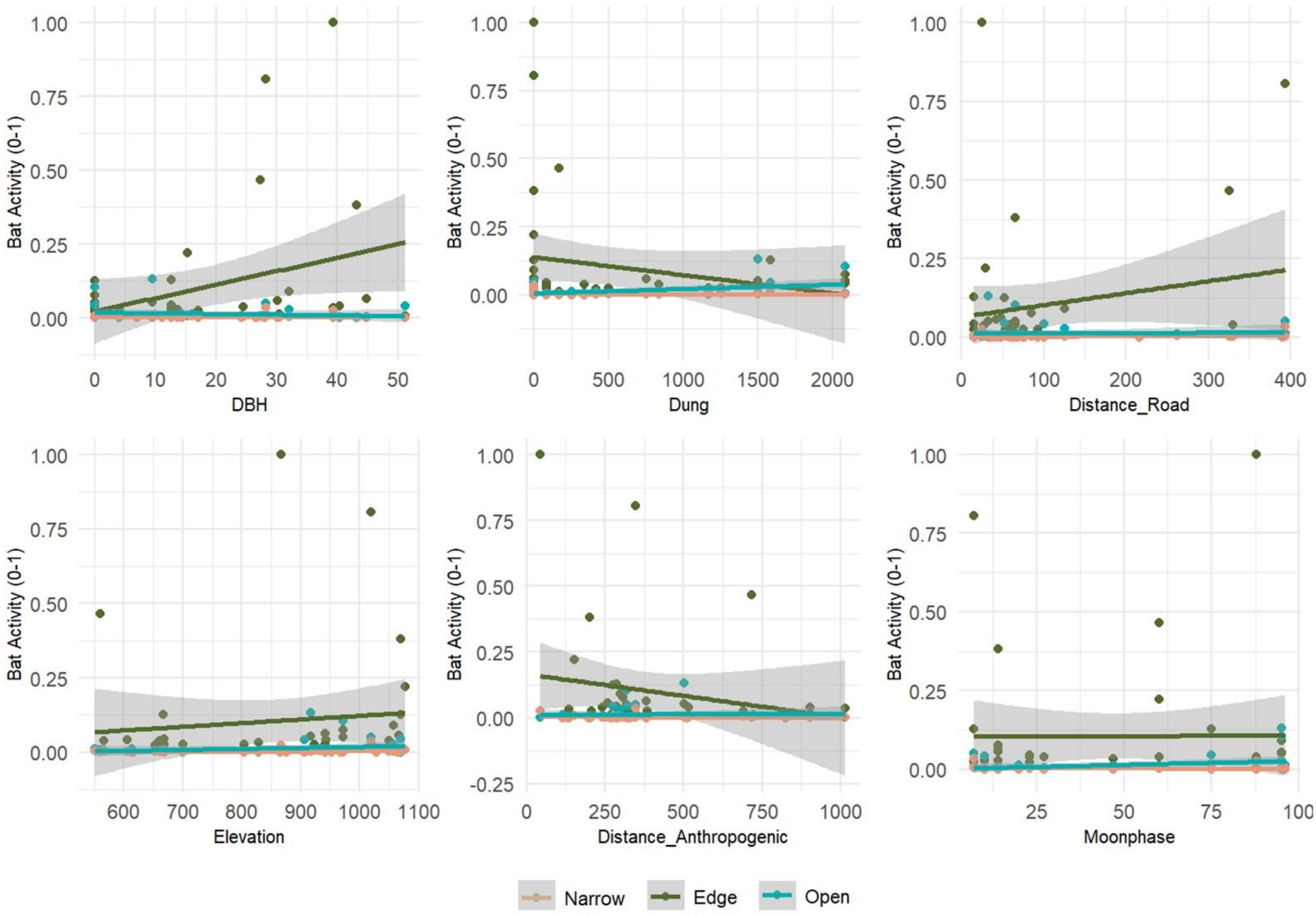


Figure 24. Relationship between bat activity of different foraging guilds with vegetation structure, landscape scale and climatic variables.

Effect of vegetation structure and landscape scale structure

The generalized linear mixed model showed different relations between habitat structure features, vegetation structure, landscape scale variables, and species bat activity. Overall, a weak association was observed for all significant variables and intercepts. On the general scale, closer distances to anthropogenic structures ($p<0.05$), closer roads, large openings, and habitats with higher dung density and larger trees significantly promote overall bat activity. Similarly, edge bat activity responded negatively to the distance to anthropogenic structures ($p<0.05$) but increased activity with higher elevations ($p<0.05$). Narrow bat species showed potential increased activity near unpaved roads and higher bat activity at higher elevations. Open bat species displayed higher activity with proximity to unpaved roads and higher dung density ($p<0.05$), while open species tend to be more active in areas with smaller trees ($p<0.01$) (**Table 6**).

Table 6. Summary of the selected GLMM models for all bat species activities, open, edge, and narrow foraging guilds activity using habitats and season as random variables and a different combination of vegetation structure and landscape scale as fixed variables. '.'p < 0.1; *p < 0.05; **p < 0.01; ***p <0.001 'n.s' not significant.

GLMM	Parameters	Estimate	Standard Error	Error Z	Value P
	Intercept	2.1232	0.667	3.182	**
All bat activity	Distance anthropo	-0.023	0.008	-2.829	**
AIC: 338.1	Distance road	0.033	0.002	0.015	*
	Dung density	0.010	0.003	2.64	**
	Mean DBH	0.046	0.016	2.727	**
Edge bat activity	**Intercept**	0.535	1.438	0.372	.
AIC: 284.3	Distance anthropo	-0.021	0.009	-2.245	**
	Elevation	0.029	0.012	2.261	**
Narrow bat activity	**Intercept**	-3.310	1.489	-2.222	*
AIC: 103.4	Distance unpaved road	0.0031	0.016	1.847	.
	Elevation	0.0026	0.014	1.806	.
Open bat activity	**Intercept**	-0.822	0.791	-1.04	n.s
AIC: 175.3	Distance unpaved road	0.0042	0.001	2.338	*
	Dung density	0.0014	0.004	2.893	**
	Mean DBH	-0.035	0.019	1.865	.

4.4 Discussion

Bat assemblage season and habitats

We aimed to comprehend the overall influence of the habitat type, vegetation structure, landscape scale structure, and climatic variables on bat community activity and species composition across three seasons. From 10957 bat calls, we identified a total of 15 species/phonic groups. However, we did not observe any significant differences in bat activity or species richness across the four distinct studied habitats. The same pattern was also present when analyzing separately the different foraging guilds specific. Indeed, such lack of relationship seems to occur also inside the same broad vegetation type, once Węgiel et al. (2023) found no spatial evidence in bat species assemblage or activity across different forest habitats. In any case, this unclear association was also found in other studies across the Mediterranean. For example, Mendes et al. (2014) found that bat species composition and activity were similar across 8 different habitats, although seasonal variations were found. Our results are in alignment with several studies suggesting that bats forage and explore various habitats, especially in natural environments where diverse habitat types are intertwined and close to each other. Bats in heterogeneous and interconnected habitats seem to profit from the proximity of different land covers which may lead to less preference towards one type of habitat (Krings et al., 2022). In this sense, further research must be done to test whether inter-habitat differences might become relevant in other regions where forest cover is almost absent.

While no differences were detected across habitats, a significant seasonal preference was obtained concerning bat activity and species richness. Higher activity was observed during the summer season compared to the autumn and spring seasons. This seasonal trend can be highly due to an increase in foraging by pregnant and lactating females, a period with the highest energetic needs (Dietz et al. 2009). This seasonal synchrony was also highlighted by Faria et al. (2022) who found a higher bat activity peak during the summer season and suggested the presence of a strong link between bat activity and their phenological stages. Another reason for this higher activity can also be explained by a higher insect abundance during the summer season (Charbonnier et al., 2016; Meyer et al., 2016).

Effects of vegetation structure and landscape scale

Understanding how vegetation and landscape scale features can shape bat and foraging guild activity is essential for the implementation of any future conservation measurements and practices. To accomplish this, we considered a total of 15 vegetation structure and landscape scale variables. Despite the presence of several factors, our findings indicated that, on a community scale, only unpaved roads, anthropogenic structure, dung density, and DBH were significant.

The local patterns of foraging guilds groups' activity appear to be driven differently by various mixes of vegetation structure and landscape scale variables. Overall, bats showed higher dependency on landscape scale rather than the vegetation variables themselves. Such outcomes are in accordance with other studies (Charbonnier et al., 2016; Meynard et al., 2014) which reported that bat communities are more dependent on the surrounding landscape than stand scales features. The same trends were also found in tropical areas, where Falcão et al. (2018) found that bats richness and abundance were only significantly related to distance to late-successional forests, regardless of several vegetation structural variables. Such an unclear relationship between bat activity and vegetation structure seems to be the rule also in other conditions. Regnery et al. (2013) found that variations in microhabitat diversity were the only predictors explaining the abundance and community response of bats in relation to 8 vegetation structural and scale variables. In a study in the boreal forests of Finland, Vasko et al. (2020) found that only canopy cover and forest age were significant predictors of the occurrence of *Eptesicus nilssonii* and *Myotis* spp., respectively, while Mendes et al. (2017) found contrasting effects of local and landscape variables in bats site occupancy bats in a tropical savanna.

Among the assessed vegetation structure, DBH was found to be a key driven variable in affecting bat activity. Based on the multicollinearity test, a larger DBH can reflect a habitat with higher trees, more abundant microhabitats, and higher canopy cover. On several occasions, larger DBH was identified as a positive driver for bat activity as they support a higher number and diversity of TreMs which in turn can serve as roosting habitats and cavities for forest-dwelling bats (Novella-Fernandez et al., 2022; Asbeck et al., 2019; Kozák et al., 2018). Our findings were similar to those obtained by Allegrini et al. (2022) who highlighted the significant impact of higher DBH in pine plantations in increasing bat activity overall. In the same trend, Węgiel et al. (2023) indicated the positive effect of larger trees on bat activity.

As we expected, open species guild activity was negatively influenced by higher DBH. Both *T.teniotis* and *Nycatlus* species are more likely to forage in open spaces and less cluttered areas and require relatively large spaces for efficient flight and foraging. This result is in accordance with (Alder et al., 2021) who suggest that open species such as *Nyctalus* spp. tend to avoid foraging in habitats with larger trees.

As to landscape scale variables, distance to unpaved roads, distance to anthropogenic structure, and dung density stand out as the most significant variables influencing overall bat activity. Our findings showed a positive association between bat activity and the proximity of anthropogenic structures. These findings align with those reported in previous studies. For instance, Voigt et al. (2016) determined the importance of human structures as potential mating roosts for several species. Rachwald et al. (2021) found that anthropogenic structures have a positive impact on forest bats as they can serve as additional roosting sites, particularly for synanthropic, cave-roosting, and species that do not typically occupy tree cavities. Given that most of these structures are situated at low elevations, synanthropic and thermophilic species such as *M. myotis* and common pipistrellus could potentially benefit from these structures, especially during winter seasons. Our findings support these hypotheses, as the distance to buildings was found to positively influence the activity of edge species, including *P. kuhlii* and *P. pipistrellus.* As shown, the presence of such structures can greatly benefit non-forest dwelling species. However, it is crucial to maintain a limited number of anthropogenic structures and activities, as an excess of such elements may have an overall negative impact on bat community.

On the other hand, unpaved roads were found to be positively associated with bat activity, particularly among open bat species such as *T. teniotis*, *Nyctalus* spp., and *E. serotinus*. In this scope, Altringham and Kerth, (2016) suggest that these roads may serve as open gaps amidst dense forests, providing permanent corridors and routes for such bats to navigate and forage. In another study, Piksa et al. (2022), also found a relevant role of these roads in increasing bat activity, especially for species that typically avoid cluttered areas, including *Nyctalus* spp., and *E. nilssonii*. This was also illustrated by the results obtained by Rachwald et al. (2021) who found a positive influence of these roads on open guild species. In the United States, Zimmerman and Glanz, (2000) observed higher bat activity in the presence of unpaved roads, and similar results were also found by Hein et al. (2009), where bat activity was five times higher in the presence of roads. Moreover, our results are confirmed by those obtained by Bellamy et al. (2013) who found that higher road density can increase bat activity of narrow-adapted species including *Myotis* and *Plecotus* species. Future research is needed to understand whether paved roads across natural areas different may have implications for bat activity compared to unpaved roads.

The influence of cattle activity and dung density on bat activity has already been explored in many studies. Our findings align with those of Ancillotto et al. (2017), who underlined a positive effect of free-ranging cattle on bat activity. These results were furtherly supported by Ancillotto et al. (2021), who observed that free-ranging cattle offered foraging opportunities for *Pipistrellus* and *Hypsugo* species. However, on a guild-specific level, our findings diverged from these results. Contrary to the previous findings by Ancillotto et al. (2017), our study revealed that dung density favored the bat activity of open species rather than edge species. These results can be explained by the concentration of free-ranging activity in open areas and shrubland habitats. Theoretically, the positive influence of dung density is generally explained by the likelihood that cattle dung supports higher insect biomass, particularly Coleoptera and Diptera (Downs and Sanderson, 2010). Throughout our analysis, we also included the distance to the forest edge among predictors, as it is a significant landscape scale variable influencing bat activity in many studies. However, contradictory to (Frey-Ehrenbold et al., 2013) and (Treitler et al., 2016), we did not find any significant effect of the proximity of forest edge on bat activity. Similar contradictory results were also obtained by (Ewert et al., 2023) who did not find any impact of forest edge on bat activity.

Different bat species and foraging guilds exhibited distinct responses to various landscape and vegetation structure complexity. This variation may be explained by the different foraging strategies, wing morphology, and echolocation call structure. We found that comprehending bat responses cannot be presumed based on the response of a single foraging guild or species. This complexity necessitates the consideration of unconsidered factors that may play a crucial role in driving bat activity and species compositions. For instance, expanding the buffer zone could offer a more representative perspective and may better reflect the impact of the mosaic landscape scale on the spatial dynamics of certain mobile foraging species. Although our current study did not account for nocturnal insect abundance, future research should investigate the influence of such factors on bat activity. Further research should delve into the collective impacts of distinct vegetation and landscape characteristics within each specific habitat.

Climatic influence

Elevation gradients are known to influence the overall composition and distribution of bats (Caprio et al., 2020). With the ongoing climate change events it is anticipated that bats will shift toward higher altitudes or latitudes as indicated by COST Action CA18107 (2023) last report. Regarding species-specific responses, other climatic factors appear to influence bat activity.

We observed an elevation-associated increase in bat activity, contrary to findings in many related studies. In Greece (Georgiakakis et al., 2009) recorded fewer bat passes at higher elevations compared to low and medium elevation ranges. Similarly, in South Africa, (Weier et al., 2017) discovered a negative association between bat activity and higher elevations. Furthermore, it's important to recognize that regions at higher elevations experienced less human disturbance and exhibited greater forest cover, which can also be a driven factor for this higher activity. The impact of moon illumination intensity remains controversial and can vary depending on different factors (Appel et al., 2021). Despite that higher illumination nights can impose a significant predation risk on bats and reduce their activities (Bhalla et al., 2023). Here we found that bat species like *T.teniotis* were positively influenced by higher moon illumination. This finding is consistent with the previous hypothesis obtained in suggesting that open foraging species tend to shift their activities from above forests to more open fields during moonlit nights (Roeleke et al., 2018). Our results align with those of (Kolkert et al., 2020) who observed an increase in overall bat activity during nights with greater moon illumination intensity, explaining the facts that bats can increase their detectability of prey and capturing rates (Roeleke et al., 2018).

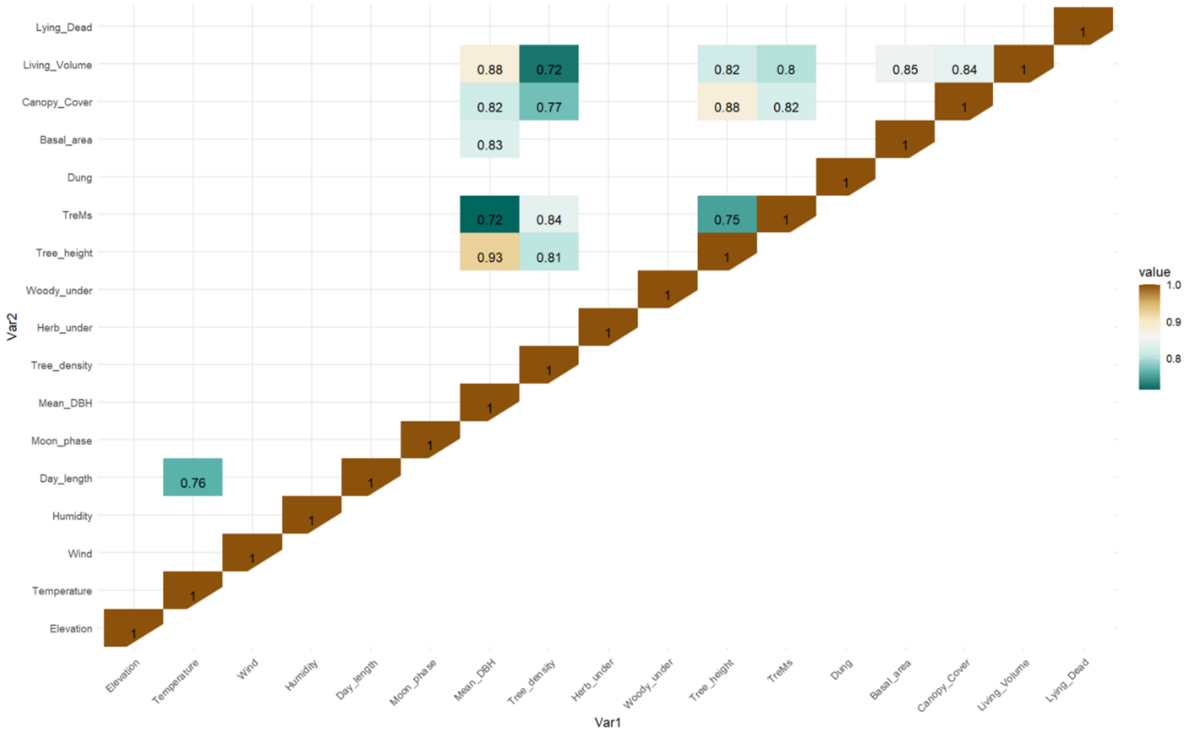
Nightly bat activity pattern

Nightly bat activity exhibits diverse activity patterns and varying numbers of peaks among different species. Existing literature suggests that substantial interspecific differences in activity patterns arise from multiple factors, such as foraging habitat seasonality (Ignaczak et al., 2019), the annual cycle period for reproduction, and insect abundance (Salvarina et al., 2018; Weinbeer et al., 2006). For instance, we documented a unimodal activity pattern for *E. serotinus* species. However, Catto et al. (1995) demonstrated that *E. serotinus* activity is unimodal but can become bimodal during pregnancy, with a prominent peak after dusk and possibly a smaller one before dawn. Our findings indicate that both *P. pygmaeus* and *T. teniotis* exhibit bimodal nocturnal activity patterns. In contrast, (Mas et al., 2022) in Spain described their activity as erratic for *T. teniotis* and unimodal for *P. pygmaeus*. Whereas *M. schreibersii* exhibited a bimodal activity pattern, aligning with observations made by (Schaik et al., 2015) and (Ignaczak et al., 2019) (SI E).

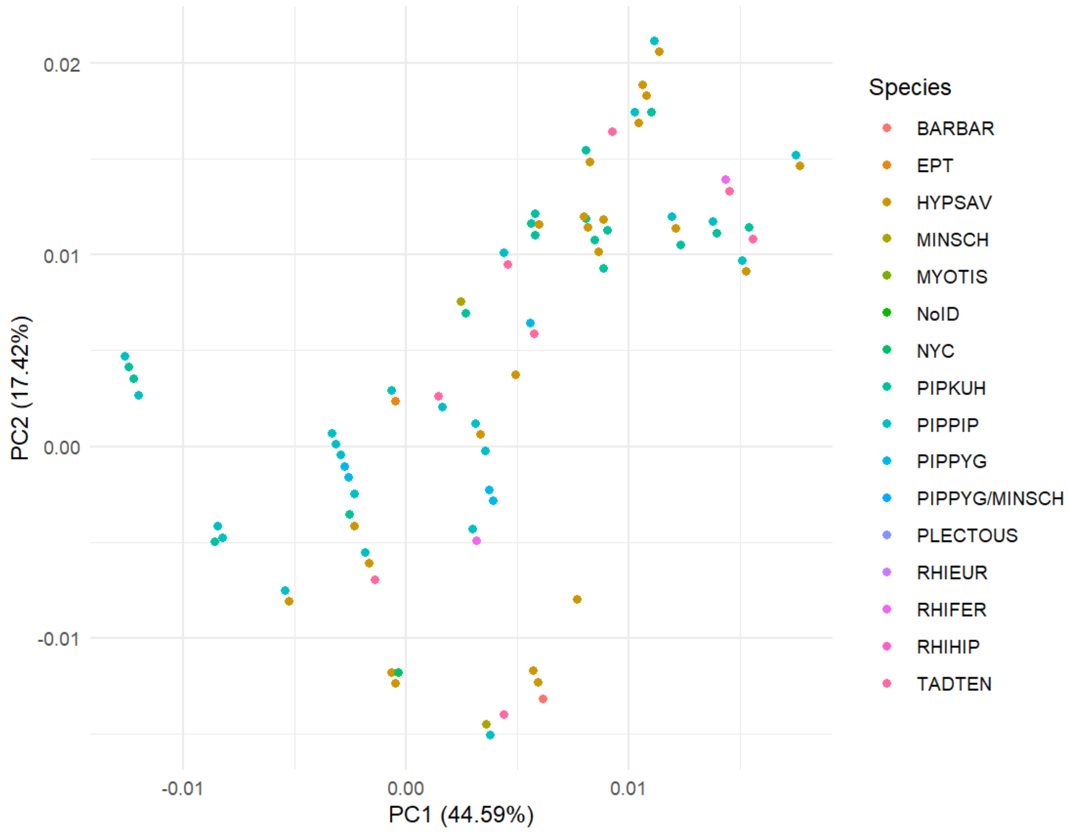
Acknowledgements:

We would like to thank Dr. Emilio Badalamenti and Giovanni Giardina for their efforts and assistance with fieldwork and data collection. We also thank Giovanna Sala for providing access to the climatic data. We also thank the Sicilian Dipartimento Regionale dello Sviluppo Rurale e Territoriale for their logistical support.

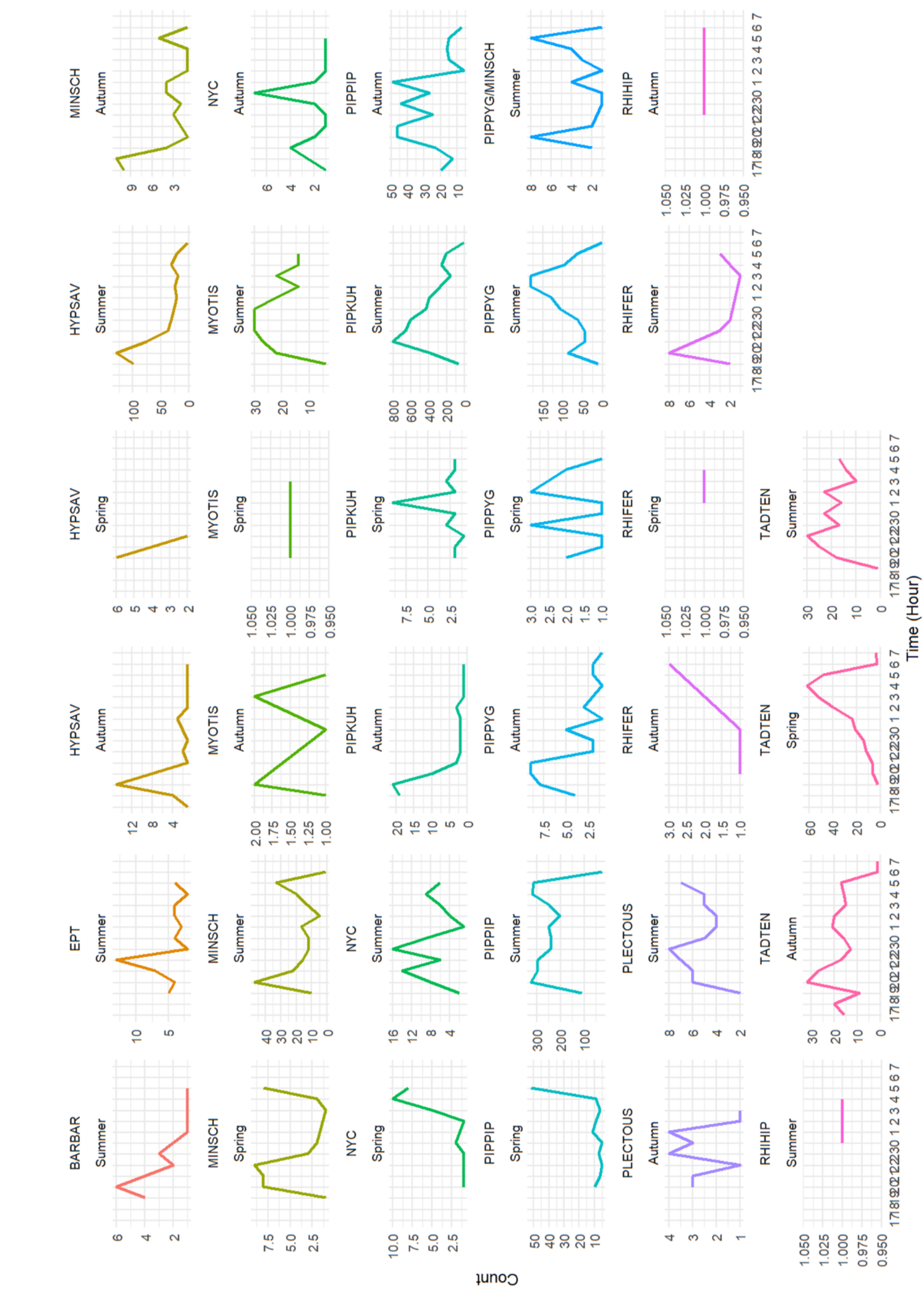
Supplementary information



SI C. Spearman correlation across the selected variables.



SI D. PCA of the recorded species.



SI E. Bat species nightly activity patterns across the seasons.

Chapter 5

Newly reported species across Sicilian archipelago: A sporadic acoustic monitoring of insular bats

Abstract

The exploration of bat fauna on the Sicilian archipelago is limited. In 2023, we conducted sporadic acoustic monitoring across six islands. We identified a total of nine distinct species/genus, with *P. kuhlii* being the most prevalent. Some taxa were observed for the first time, including *Nyctalus* spp. in the Aeolian islands, *H. savii* and *P. pipistrellus* in the Pelagie Islands (Lampedusa), and *T. teniotis* in Pantelleria. Our findings suggest a potential lack of research on bat assemblage in many of these islands, highlighting the need for more monitoring programs for future bat conservation.

Keywords: Sicily- bat- acoustic monitoring- conservation

5.1 Introduction

Islands, comprising less than 8% of the world's land area, are renowned for harboring numerous endemic species, unique taxa, and diverse ecological niches (Russell & Kueffer, 2019). However, with over 40% of the world's highly threatened terrestrial vertebrates residing on islands, insular ecosystems are exceptionally vulnerable to ongoing Anthropocene defaunation (Spatz et al., 2017). Islands are considered highly sensitive habitats, greatly impacted by historical and present anthropogenic and environmental changes, rendering insular bat species particularly susceptible to conversions and threats (Conenna et al., 2017; Spatz et al., 2017; Jones et al., 2009). Bats are the sole mammalian representatives in island ecosystems, playing a significant role in sustaining these fragile ecosystems (Jones et al., 2009). Of the over 1,400 extant bat species, approximately 60% occur on islands, with around one-quarter of all species being insular endemics (Jones et al., 2010; Conenna et al., 2017).

The Sicilian archipelago comprises several smaller islands clustered around the main island. Each island forms an isolated ecosystem, resulting in a diverse species complex composition and assemblage. Earlier evidence indicated that these islands were promising hotspots for some bat species (Bonaparte, 1833). However, these areas remain understudied, with most research dating back to the mid-to-late 20th century, except for recent studies conducted in Pantelleria by Ancillotto et al. (2020) and Fichera et al. (2022). A recent review by Massaad et al. (2023) suggested additional effort and monitoring of these islands to address the gaps and scarcity of updated data across; Aeolian Islands (Masseti and Zava, 2021; Zava et al., 1994; Fiore et al., 1992; Kahmann, 1957), Egadi Islands (Masseti and Zava, 2021; Fornasari et al., 1997; Zava and Lo Valvo, 1991; Felten and Storch, 1970), and Pelagie Islands (Fornasari et al., 1997; Zava et al., 1994; Kock, 1989; Zava and Catalano, 1983; Felten and Storch, 1970).

Here we aim to contribute to the understanding of bat species distributions across six distinct islands surrounding Sicily, obtained through sporadic acoustic monitoring. We hypothesize that the composition of bat species will display significant variation among the islands, reflecting a correlation between bat species richness and island biogeography features.

5.2 Materials and Methods

A sporadic acoustic survey was conducted from August 2022 till July 2023 across six different islands namely, Aeolian Islands (Lipari and Salina), Egadi Islands (Levanzo), Pelagie Islands (Lampedusa), Pantelleria, and Ustica. A total of 38 sampling sites were monitored during the autumn, spring, and summer seasons. The sampling sites represented a variety of habitats (urban areas, forest, open space, agricultural areas, and riparian areas) (**Table 7**). Bat detectors (AudioMoth 1.2 version) were employed one night in each sampling site. Bat detectors were placed 1.5 m above the ground and set to record from dusk until dawn 5s each minute.

All the calls were analyzed using Kaleidoscope software and then manually rechecked based on the descriptive call characteristics of Italian bat calls by Russo and Jones (2002). In most cases, bat calls were assigned to the species level, unless there was an ambiguity. This the case of calls from *Pipistrellus pygmaeus* and *Minotpterus schreibersii* which were classified as *Pipistrellus pygmaeus/Miniopterus schreibersii*. *Nyctalus* and *Plecotus*, on the other hand, were identified at the genus level. A bat pass was considered for a sequence with at least two distinct pulses, and a feeding buzz was also noted.

Table 7. Summary of the studied Islands (Lampedusa, Lipari, Levanzo, Pantelleria, Salina, Ustica), size, distance to nearest mainland, habitats (open space- urban- forest- caves- mix woodland- vineyard- pine- shrubland- olive groves- rocky cliff- riparian), elevation range (min-max), number of sampled sites per island, and monitored seasons (autumn-spring-summer).

Island	Size Km^2	Distance to mainland Km	Habitats	Elevation range (m.a.s.l)	Number of sampling sites	Monitored season
Lampedusa	20.2	205	open space- urban- forest- caves	6-120	11	autumn- spring- summer
Lipari	37	30	shrubland- olive groves	52-96	2	summer
Levanzo	5.82	14	pine forest	62	1	summer
Pantelleria	84.53	70	open space- Mix woodland- vineyard- pine forest and shrubland	8-350	12	autumn- spring- summer
Salina	26.1	38.2	urban- open space- vineyard- olive groves- mix woodland	12-341	8	spring- summer
Ustica	8.65	53	riparian- open space- mix woodland	13-101	5	spring- summer

5.3 Results

A total of 870 bat passes and 63 feeding buzzes were recorded belonging to nine different species and genus. An average of 18.1 bat passes per night was recorded across the island. Lipari had the highest spring/summer bat activity per night with 117 bat passes followed by Lampedusa 55.2, Pantelleria 16.7, Ustica 11.7, Levanzo 9, and Salina 6 passes (**Figure 25A**; **Table 8**).
Among the recorded species, *P. kuhlii* was the most recorded species with 571 passes (65.8% of the recorded calls), followed by *H. savii* 229 passes (26.3%), *P. pipistrellus* 26 passes (3%), and *T. teniotis* 24 passes (2.9%). To a lesser extent, we recorded more elusive species such as *Plecotus*. spp 7 passes (0.7%), *P. pygmaeus/M. schreibersii* 6 passes (0.68%), *R. hipposideros* two passes (0.28%), *R. ferrumequimum* one pass (0.14%), and *Nyctalus*. spp two passes (0.23%). Seven bat species were detected in Lipari, and Salina, five in Pantelleria and Ustica, three in Lampedusa, and only one species in Levanzo (**Figure 25**; **Table 9**).

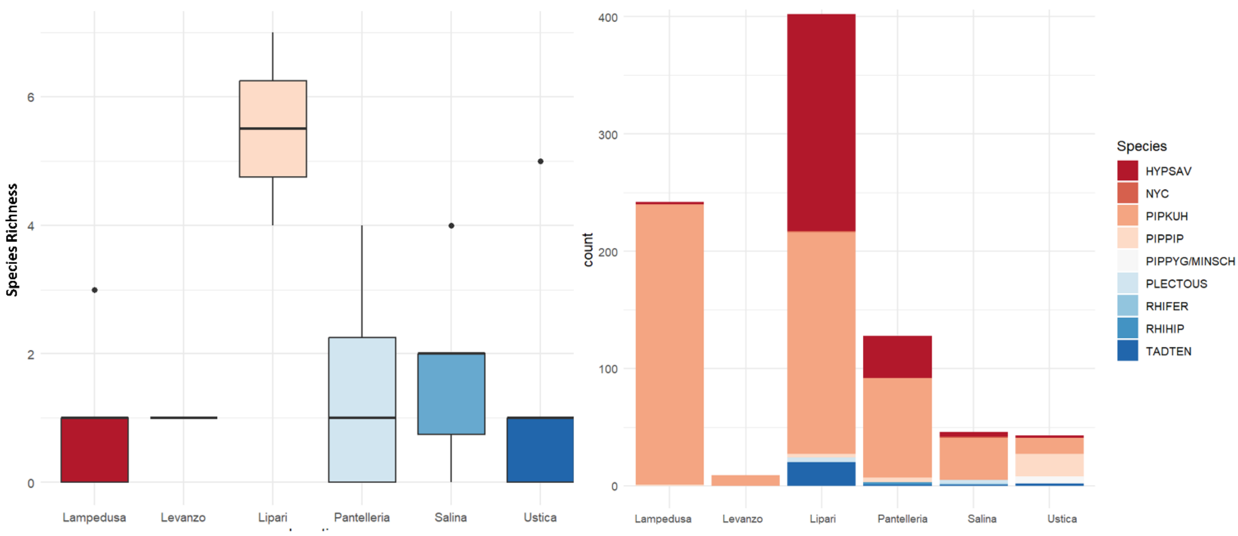


Figure 25. A: Bat species richness across the six studied islands. B: Bar plot depicting the distribution of bat species calls recorded by species across the studied islands.

Table 8. Total number of recorded bat passes and feeding buzzes across the six studied islands.

Island	Total Bat passes	Bat passes/night (summer season)	Total Feeding Buzz
Lampedusa	242	55.2	8
Levanzo	9	9	0
Lipari	402	117	41
Pantelleria	128	16.7	9
Salina	46	6	5
Ustica	43	11.7	0
Total	**870**		**63**

Table 9. Distribution of recorded bat species across the studied islands. Recorded species is indicated by (1), and absence by (0).

Species	Lampedusa	Levanzo	Lipari	Pantelleria	Salina	Ustica
H. savii	1	0	1	1	1	1
Nyctalus spp	0	0	1	0	1	0
P.kuhlii	1	1	1	1	1	1
P. pipistrellus	1	0	1	1	0	1
P. pygmaeus/M. schreibersii	0	0	0	0	0	1
Plecotus spp	0	0	1	0	1	0
R. hipposideros	0	0	0	1	0	0
R. ferrumequinum	0	0	0	0	1	0
T. teniotis	0	0	1	1	1	0
Total	**6**	**1**	**6**	**5**	**6**	**5**

5.4 Discussion

Species compositions of bat assemblage varied among the studied islands. This confirms the island's uniqueness and distinctive ecosystem. The presence of nine bat species/genus was confirmed throughout this study using acoustic monitoring conducted occasionally throughout the year. We recorded the following species: Common pipistrelle, *Pipistrellus pipistrellus* (Schreber, 1774); *Kuhl's pipistrelle*, *Pipistrellus kuhlii* (Kuhl, 1817); Savi's pipistrelle, *Hypsugo savii* (Bonaparte, 1837); European free-tailed bat, *Tadarida teniotis* (Rafinesque, 1814); Greater horseshoe bat *Rhinolophus ferrumequinum,* Lesser horseshoe bat, *Rhinolophus hipposideros* (Borkhausen, 1797), *Nyctalus* species *and Plecotus* species. Among the 26 species reported across Sicily (Massaad et al., 2023), only nine were recorded on the small island in our study.

P. kuhlii was the most predominant species and was recorded across all the studied islands. Some species, such as *Nyctalus* spp. were reported for the first time. The presence of this species, in our opinion, can be explained reasonably by the fact that *Nyctalus* is a well-known migrant bat species that travels across the European continent (Hutterer et al., 2005). Here is a short overview of the current and previously reported species across the studied islands:

Aeolian islands (Lipari and Salina)

In our study, we identified several new taxa that had not been previously documented on any of the Aeolian islands. For instance, the *Nyctalus* species was observed for the first time on both Lipari and Salina. The distinction between *Nyctalus* species and T. *teniotis* was based on the call end frequency, with *Nyctalus* emitting sounds approximately 21 kHz higher than those produced by *T. teniotis* around 13 kHz. Additionally, the presence of *Plecotus* spp. was documented for the first time in the Aeolian islands. With these findings, the total number of bat species identified across the Aeolian islands now stands at a total of seven bat species.

As to other species, our results confirm again the presence of *P. kuhlii*, *T. teniotis, R. ferrumequinum*, and *H. savii* species, which were previously reported by (Zava et al., 1994; Fiore et al., 1992; Crucitti and Tringali, 1985; Kahmann, 1958). However, *M. blythii* was not detected, although reported in previous studies.

Ustica

This is the first record of *P. pygmaeus/M. schreibersii* across the Ustica islands. However, the absence of any social calls posed a challenge in the identification of the species. Still, we tend toward the likelihood that the detected species is *P. pygmaeus* rather than M. *schreibersii*, considering the earlier documentation reporting its presence at the end of the 19th century (Agnelli et al., 2009). Conversely, the other recorded species (*H. savii*, *P. kuhlii*, and *P. pipistrellus*) were also reported by Zava et al. (1994) and Fornasari et al. (1997). Despite being reported in earlier studies (Zava et al., 1994), no calls of *E. serotinus* were detected during our monitoring efforts.

Pelagie island (Lampedusa)

Two previously undocumented species, including *H. savii* and *P. pipistrellus*, were recorded for the first time on the island. Despite the ongoing seasonal monitoring, *M. schreibersii* and *M. myotis* were not found, contrary to previous reports (Kock, 1989; Zava and Catalano, 1983; Felten and Storch, 1970). Only *P. kuhlii*'s presence aligned with earlier reports (Toschi, 1970).

Pantelleria

In addition to identifying previously reported species such as *H. savii*, *P. pipistrellus,* and *P. kuhlii*, we documented a newly reported species, *T. teniotis*, for the first time in Pantelleria. Notably, despite the recent studies and bat sampling conducted across the islands in the last few years (Fichera et al., 2022), this marks the first recording of *T. teniotis* in the region. As to our knowledge, *T. teniotis* was most likely undetected in previous mist netting-based studies due to its behavior, as *T. teniotis* generally flies at high level from the ground (Pejić et al., 2017). Standard mist netting frequently misses such species, which are more likely to be detected through acoustic monitoring (O'Farrell and Gannon 1999). Furthermore, a single *R. hipposideros* bat pass was recorded across the islands, which was previously reported as *R. hipposideros*. minimus subspecies by Felten and Storch (1970), and recently as *R. hipposideros* by Fichera et al. (2022). A colony of approximately 30 *R. hipposideros* was observed roosting in an old house in Pantelleria **(Figure 26**). The inclusion of newly recorded species, in addition to those reported by Fichera et al. (2022), brings the total number of bat species in Pantelleria to eight species (SI F).

Figure 26. *R. hipposideros* colony observed in an abandoned house in Pantelleria (Photo by Tommaso La Mantia).

Egadi islands (Levanzo)

Our findings are in alignment with those previously conducted by Zava et al. (1994), who also reported the presence of *P. kuhlii*. However, we could not confirm the presence of *T. teniotis* across the island. It must be due to the weak sampling effort and limited number monitored night. As monitoring only took place for just two nights during the summer season.

Our findings may provide biased information about species' presence and distribution across the studied islands due to uneven sampling efforts, and small sampling size which can potentially affect species detection. However, except for Pantelleria, this brief communication can provide first evidence and an updated date for most of the islands. Another point to highlight was the uneven sampling effort across the season, as the summer season was expected to have higher activity and more records of bat passes and species. For instance, in Lipari, all sites were sampled only during the summer season, whereas on other islands, some monitoring was done during the autumn or spring seasons. This was reflected in the difference in bat activity detected across several islands. To note, Stromboli Island was also monitored for two nights (Ginostra-summer), but no bat calls were recorded.

Our findings underline the necessity of revising more profoundly the understanding of bat community composition of bats across the Sicilian archipelago. Despite the promising results, it can still be considered uncertain as it was exclusively identified through acoustic monitoring. Hence, for better species identification and conservation we urgently recommended multi-proxy techniques including mist netting, long-term roost monitoring, and DNA sampling especially as sometimes relying on just one method can result some unbalance and uncertain species identification.

The unique treatment of the island's ecosystem and biogeography lies in the distinctiveness of insular bats and their dynamics varying across each island (Russell & Kueffer, 2019). When examining species compositions among the various islands, factors such as quantitative considerations, including distance from population sources and area distance to the mainland, can all influence bat species composition. A diverse species assemblage compositions was obtained, with notable distinctions observed in Pantelleria and Lampedusa. This discrepancy could be linked to their considerable isolation, as both islands are the farthest from the mainland compared to the other studied islands. In contrast, neighboring islands in the Aeolian archipelago tend to exhibit similar bat species assemblages, albeit varying in terms of abundance and activity. Further research, conducted through comprehensive sampling and employing a multi-approach methodology, should carefully consider the influence of these factors on both species' composition and the species-area relationship.

The presence of potentially new recorded species, as well as new distribution records of bats, highlights the importance of conservation, especially on islands where there are emerging and various threatening factors affecting bats. In this sense, we would like to highlight the socially underestimated threats that cats may pose (Oedin et al., 2021; Salinas-Ramos et al., 2021). Unfortunately, many free-roaming and community cats were observed throughout the small islands (personal observations) (**Figure 27**). We recommend improved management of free-ranging cats, which are regarded as a major threat to these insular bat species (Lepczyk et al., 2023).

Finally, future research efforts should be suspended to cover less studied islands, particularly those known to be less anthropogenically disturbed, such as Filicudi and Alicudi.

Figure 27. Free-roaming cats captured across the street of Lipari Island (Captured by Mark Massaad 5/07/2023).

Acknowledgement:

We extend our gratitude to Dr. Camillo Cusimano, Dr. Dario Scuderi, and Frederico Priola for their assistance in conducting field surveys and deploying the bat detectors.

Supplementary information

SI F. All time recorded and reported bat species across the following islands.

Island	Species	References
Aeolian islands (Salina, Lipari)	P. *kuhlii; H. Savii; T. Teniotis; M. blythii, R. ferrumequinum; P. pipistrellus; Nyctalus spp; Plecotus spp.*	(E.H Giglioli 1878, Kahmann, 1958, Crucitti and Tringali, 1985; Fiore et al., 1992; Zava et al., 1994, Massaad et al., unpublished material)
Pelagie island (Lampedusa)	P. *kuhlii;* H. *savii;* and P. *pipistrellus; M. myotis;* M. *schreibersii*	(Felten and Storch, 1970; Toschi, 1970; Zava and Catalano, 1983; Kock, 1989, Massaad et al., unpublished material)
Pantelleria	P. *austriacus*, P. *pipistrellus*, R.*hipposideros* P. kuhlii, P. *gaisleri*, M. *punicus*, R *mehelyi*, T.*teniotis*	(Fichera et al., 2022, Massaad et al., unpublished material)
Egadi islands (Levanzo)	P. *kuhlii;* T. *Teniotis*	(Zava et al., 1994; Massaad et al., unpublished material)
Ustica	*Nyctalus* spp. P. *pygmaeus/*M. *schreibersii,* P. *kuhlii,* P. *pipistrellus/*P. *pygmaeus*	(Zava et al., 1994; Massaad et al., unpublished material)

Chapter 6

General Discussion

Systematic review as a tool to uncover research gaps

The term "filling the gap" involves adding necessary elements to something to achieve completeness. Bat conservation is one of the top global concerns for research today; however, conservation efforts may still be limited and impotent due to inadequacy and scarcity of data. Hence, identifying research trends, gaps, threatening factors, species status, and geographical priorities is an essential tool for future conservation, protection, and research prioritization.

In the Mediterranean region, insular bats, in particular, are classified as threatened species and are in urgent need of conservation efforts (Conenna et al., 2017; Zava et al., 1994). An effective conservation measurement necessitates the integration of research synthesis approaches. Systematic reviews are recognized for offering an evidence-based approach that can accurately reflect the state of research, revealing the gaps and priorities in an unbiased approach.

As it is mostly unclear what has been done on both ends (Mediterranean and Sicily), in the first part of this thesis our objective was to obtain a thorough overview of Mediterranean and Sicilian research status.

On the broader level, our findings from Chapter 2 confirmed the increase in the number of publications covering a diverse range of research scope related to the Mediterranean bats population over the last years. A similar trend was observed in Chapter 3 on studies conducted in Sicily. This increase was noticed mainly in research focusing bat ecology and taxonomy.

This expansion can be attributed to the advancement of methodology, especially in bat monitoring and species identification through molecular techniques and acoustic monitoring. Overall, we found 61 cited bat species where 60 were considered insectivorous and 1 as frugivorous bat. Ecological research focused mainly on the ecological role and attributes of bats as insect predators. As an example, Puig-Montserrat et al. (2015) in Spain calculated the economic benefits of bats at approximately 22.5 euros/ha per year. Bats economic contribution was also estimated in vineyards (Charbonnier et al., 2021; Froidevaux et al., 2017), cotton fields (Korine et al., 2019), and olive groves (Davy et al., 2007).

Beyond that, a substantial amount of research focused on the description of newly discovered species, cryptic species, and the description of their geographical range and distribution across the Mediterranean. Several bat species were lately identified or reported for the first time across the Mediterranean area including: *Pipistrellus sardus* (Mucedda et al., 2002), *Pipistrellus kolombatovici* (Ancillotto et al., 2019), *Myotis punicus* differentiated from *Myotis blythii* and several cases of cryptic species including *Myotis nattereri* (Puechmaille et al., 2014). On the regional level, Sicilian research appears to follow the same trend. In Chapter 3**,** we found that over the last decades, several species were rediscovered or newly reported. This included the rediscovery of *Myotis bechsteinii*, by Di Salvo et al. (2012), Soprano pipistrelle (*Pipistrellus pygmaeus*) by Fichera et al. (2012), and *Barbastella barbastellus* by Mucedda et al. (2012). Recent research by Ancillotto et al. (2020), supported by Fichera et al. (2022), highlighted the presence of a new species, Gaisler's long-eared bat (*Plecotus gaisleri*), in Pantelleria, bringing the total number of Sicilian bats to 26 species. Over the last 15 years, bat species descriptions were distinguished and have outpaced that of other mammalian species by more than 25% (Burgin et al., 2018). Yet, our reviews revealed persistent geographical biases not only across Mediterranean countries but also within each country.

This bias was evident both regionally and within the specific habitats studied across Sicily. More than 70% of the studies were carried out in the northwest (Palermo province) and southeast (Catania and Siracusa provinces) of the island.

Role of acoustic monitoring in filling highlighted research gaps

Through our systematic review and research conducted at both the local and regional levels, we identified key research gaps that require urgent implementation efforts. We observed a greater research focus on cave-dwelling bat species compared to those dwelling in forests. Another research gap was identified where only limited number of studies was dedicated to the small islands surrounding the Sicilian Island. In chapter 4 and 5, we addressed these gaps through acoustic monitoring to gain a better understanding of bats in natural habitats, as well as their distribution across the understudied islands.

Bats and vegetation, landscape scale, and climatic influence

Insular bats have become critically threatened due to ongoing anthropogenic and environmental changes. We found out that in Chapter 2, habitat disturbances, such as fires, deforestation, and disturbance, were found to be the most cited threatening factor with 18.3%, followed by climatic changes and forest disturbance. Moreover, on the local level, forest and natural habitats were considered the least studied area compared to studies conducted on riparian and cave dwelling species.

This disturbance is thought to be generally affecting bats compositions, activity, and foraging behavior. Bats are highly sensitive to environmental changes (Russo et al., 2021; Jones et al., 2009) and their distribution depends greatly on habitat structural characteristics (Froidevaux et al., 2021; Mendes et al., 2017). To further explore the impact of disturbances and habitat changes on bats, an acoustic monitoring study was carried out in the Ficuzza Natural Reserve.

In Chapter 4, we show that vegetation structure, and landscape scale can be a primary factor influencing bat activity across natural habitats. Our findings were consistent with those previously observed across the Mediterranean natural habitats. (Allegrini et al., 2022; Heim et al., 2017; Mendes et al., 2017; Charbonnier et al., 2016; Meynard et al., 2014). However, we found that bats were more influenced by landscape scale than micro vegetation structural features. Overall bats reacted differently among guild-specific levels. We observed that habitats with larger DBH had a positive impact on overall bat activity. It is believed such habitats can provide key resources for bats including, roosts and insect prey (Hendel et al., 2023; Kozák et al., 2018). Open and narrow bat species were found to benefit from the proximity of unpaved roads. Higher dung density also had positive implications, especially on open bat species. As to climatic factors, bats react differently to species-specific. Overall, general bat activity was positively associated with moon intensity and higher elevation. These results appear to be in opposition to those observed in most of the research (Bhala et al., 2023; Appel et al., 2021).

Recent research focuses on understanding how bats respond to their surroundings. However, significant controversy and variation exist in the reported influences, whether related to vegetation, landscape scale, or climatic variables. Through observations in the research, it becomes evident that there is a lack of standardized methodology and common acoustic monitoring protocol. This absence makes it challenging to compare our findings to previous studies or may lead to less comparable results. Therefore, it is necessary to establish standardized protocols and adhere to clear methodologies, especially within the same habitats and areas.

Small islands are a precious area for bat conservation

The Sicilian archipelago comprises numerous smaller islands clustered around the main island. Previous evidence indicated that these islands hold promise as hotspots for certain bat species. In Chapter 3, our findings revealed that these areas have been relatively understudied, with the most recent research dating back to the mid-to-late 20th century. Despite inconsistent monitoring, our study yielded interesting results. We documented in Chapter 5, for the first time to our knowledge, a couple of species that had not been previously reported. For instance, *Nyctalus* spp. in the Aeolian islands, *H. savii* and *P. pipistrellus* in the Pelagie Islands (Lampedusa), and *T. teniotis* in Pantelleria. However, these findings remain skeptical as we only based our identification on bat calls. Hence, additional monitoring is needed including other techniques to ensure the long-term monitoring and conservation of these insular species.

Future implications and recommendations

This study provided an ideal model and demonstrated the effectiveness of the followed complementary evidence-based approach. We believe that implementing the following recommendations can significantly contribute to the conservation of bats and improve future research studies on the regional and national level.

- On the regional level, we highly recommend multinational collaboration among Mediterranean countries in law enforcement and additional research cooperation particularly in the Asian and African part of the Mediterranean.
- Greater research efforts should be placed on enhancing continuous monitoring directed towards recently reported and discovered species.
- Additional research is needed in underexplored areas across the Mediterranean region, as well as in less studied areas in Sicily, particularly the small islands. We recommend incorporating multi-approach research techniques to monitor these areas. Also, further legal protection framework should be developed for small islands, given their crucial role as habitats for bats.
- Further research should be conducted encompassing additional habitat types, in order to understand more the interactions of each species across different landscapes. Monitored areas should be expanded to cover more protected areas including Nebrodi and Madonia natural reserves.
- The presence of structural mosaic landscape can significantly benefit bat assemblages across natural habitats and provide additional foraging areas. Protecting high-altitude areas from human disturbance is crucial, especially considering the expected shift of bats to higher elevations due to ongoing climate changes.
- We recommend a thorough understanding of the impact of free-range cattle on bat assemblages, given its prevalence in most protected areas and natural habitats across the island.
- We endorse the need for further evaluation and testing of acoustic monitoring methodologies with the objective of improving standardization and obtaining more reliable results. This includes an exploration of how biotic factors may influence recording quality and an examination of variations in bat activity detection among different bat detectors. Additionally, we propose investigating the impact of bat detector localization parameters, such as height from the ground, positioning angle, and casing, on recording and detection efficiency.

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Chapter 4

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