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Bats (Mammalia, Chiroptera) from a cave area in NW Italy

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Abstract
Caves play an important role in bat ecology, offering natural roosting and wintering sites. In the reproductive period, bats need to find an effective foraging environment away from the cave entrance, but only few studies have considered the use of foraging habitats in the immediate proximity of the caves. We provide a list of 18 bat species and 5 bat acoustic groups observed in a system of 81 caves in NW Italy. Winter censuses carried out during 17 years showed an increase of the Greater Horseshoe bat, a species of conservation concern. In the summer period this rare species was also observed foraging in nearby areas. Recording of bat calls in the summer period was performed both at cave entrances and in three foraging habitats present in the area surrounding the cave (woods, agricultural mosaic with artificial light, dark mosaic). Light mosaics and woods showed the highest foraging activity (160.3 and 72.0 pass/hour), while woods and cave entrances were accessed by the largest number of bat species. Our survey improves the inventory of bat species in a poorly sampled area, and we recommend a reduced tourism access, thereby promoting preservation of bats.

Keywords: Monte Fenera karst caves - *Rhinolophus ferrumequinum* - Vespertilionidae - extra-cave habitats.

INTRODUCTION

Although caves may be used by bats throughout all year (e.g. as thermally favourable breeding habitat), it is well known that their most important role in ecology of temperate bats is as hibernacula (Zukal *et al.*, 2017). However, even if there are a multitude of studies examining the presence of bats wintering in caves as part of bat monitoring programs (Loeb *et al.*, 2015; Collins, 2016), to date there are surprisingly few systematic investigations of bat activity in habitats nearby hypogeal sites compared to other habitats (Wieser *et al.*, 2020).
Hibernacula have a key role in the life cycle of bats (Altringham, 2011; Toffoli & Calvini, 2021) and the survival of many bat species worldwide depends on the availability of natural caves and other underground sites such as mines (Mickleburgh *et al.*, 2002) that provide relatively stable above-freezing temperature and high humidity (Zukal *et al.*, 2017). Subterranean winter temperatures are warmer and less variable further from the surface (McClure *et al.*, 2020) and are close to the mean annual surface temperature for the nearby area (Zukal *et al.*, 2017). Furthermore, hibernaculum humidity impacts overwinter survival since dry conditions may lead to reduced length of torpor bouts (Thomas & Geiser, 1997) and may stimulate arousals (Ben-Hamo *et al.*, 2013; Klüg-Baerwald & Brigham, 2017), increasing death probability. Highly stable climate conditions in caves thus promote energy-saving torpor and enable hibernating bats to overcome cold winters (McClure *et al.*, 2020; Speakman & Thomas, 2003).
Identifying and protecting hibernacula is crucial for bats conservation (Glover & Altringham, 2008) and their numbers and diversity in hypogeal environments are influenced by many factors including human disturbance, cave dimensions, structural complexity and microclimate, availability of food in the surrounding landscape, parasite and predation pressure. Historical use by bats and maneuverability of flight and interactions between species have a relevant impact on roost selection as well (Furey & Racey, 2015). Intraspecific needs and tolerance may vary geographically (Klüg-Baerwald & Brigham, 2017) and they also determine the suitability of a site. Selection of microclimate conditions by bats during hibernation is complex (McClure *et al.*, 2020) and not easily predictable.
External environments surrounding caves are equally important for bats all year round, even though foraging

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habitat preferences may vary among species. Forests provide cover for emerging bats reducing flight costs and predation risk (Verboom & Spoelstra, 1999; Coleman & Barclay, 2013) and represent a source of invertebrate prey (Kofoky *et al.*, 2006), whilst forest edges contribute to landscape connectivity enhancing the movement of species (Heim *et al.*, 2015). Scattered trees and hedgerow in farmland and prairie riparian areas harbour large numbers of insects and, consequently, they are used as foraging habitats (Lumsden & Bennett, 2005; Holloway & Barclay, 2000; Toffoli, 2016). In Mediterranean regions, wetlands constitute an essential ecosystem for bats and a higher bat foraging activity is reported near more accessible water bodies (Rainho, 2007). Anthropic environments are used by bats as well but, although they might be particularly attractive to *Pipistrellus*, *Eptesicus* and *Hypsugo* species, they are much less so to *Myotis* and *Plecotus* and they can even cause habitat fragmentation (Barataud, 2016).

While the study of the relationships between habitats and bat presence, or bat activity, has attracted much attention in a multitude of areas (Kunz & Fenton, 2003), the study of bat activity near the area surrounding the cave entrance has drawn little attention. We carried out systematic recordings of bat calls at cave entrance and three other habitat types (wood, dark open mosaic, open mosaic with light) to better understand the importance of these habitats for bats.

We examined the Monte Fenera Natural Park area, in NW Italy, which includes 81 caves (Lana & Sella, 1986). Previous biospeleological surveys conducted by Gruppo Speleologico Biellese C.A.I. (GSBi-CAI) from 1993 to 1999 reported the presence of Mouse-eared bats *Myotis myotis/blythii*, Lesser Horseshoe bats *Rhinolophus hipposideros* and Greater Horseshoe bats *Rhinolophus ferrumequinum* not only inside the major caves of the massif but also in several small cavities (Pascutto & Balestrieri, 2001). Further observations in subsequent

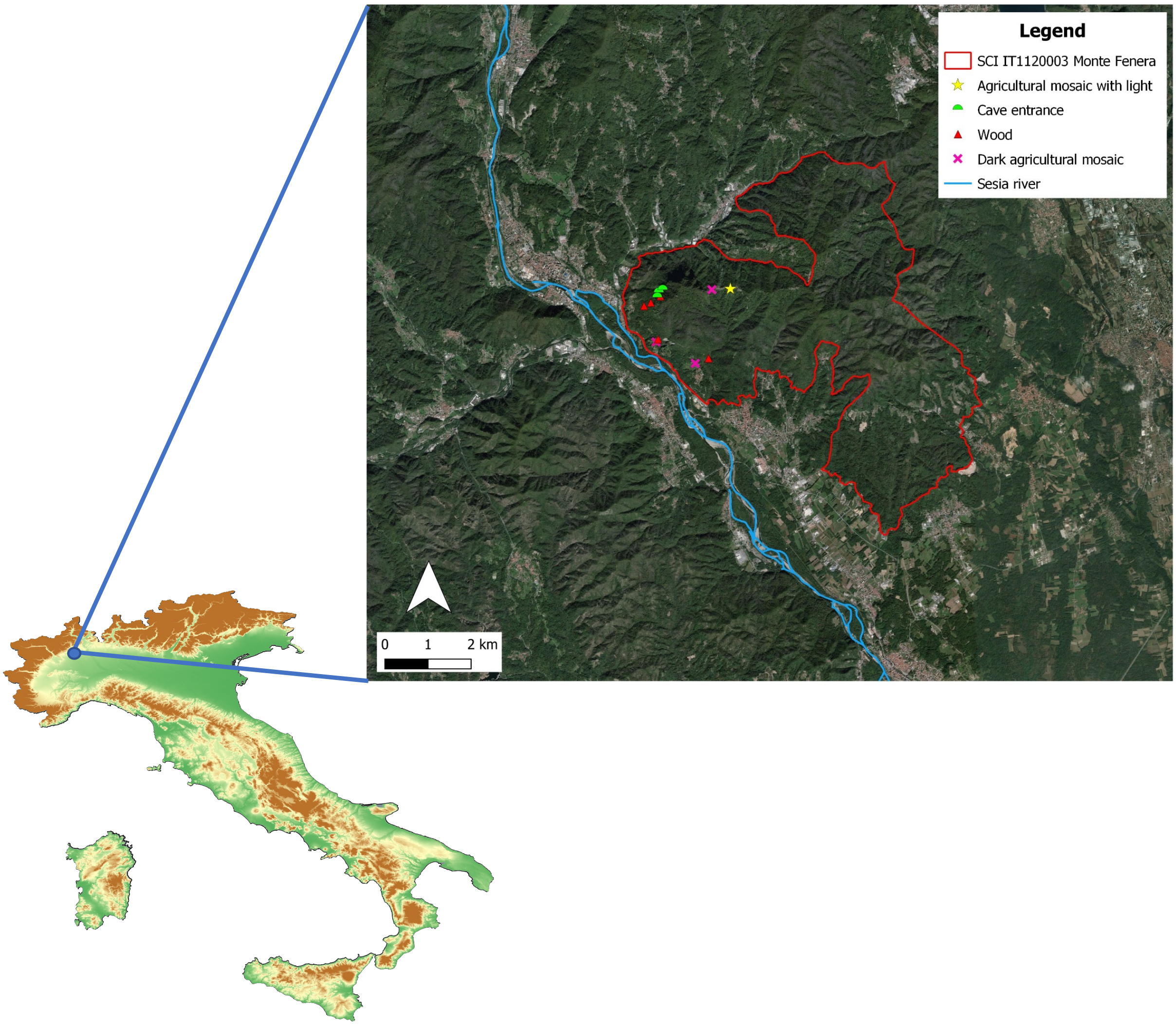

Fig. 1. Map of the study area in NW Italy. Star: sampled sites of the Fenera's caves area.

years confirmed this first list of species and revealed the attendance of a cave by Long-eared bats (Lana *et al*., 2021). The availability of different habitat types nearby caves makes the area suitable as breeding, hunting or swarming sites not only for cave-dwelling bats, extremely favoured by the geological features of Monte Fenera, but for tree-dwelling bats as well.

Our study aims to fill the lack of data about bat fauna in this area by utilizing winter censuses carried out along a 17-year period, some captures with mist nets at cave entrance, several occasional reports, and an intense monitoring through bat call recordings collected in 12 sites pertaining to four habitat categories inside the Fenera area.

MATERIAL AND METHODS

Study Area

The Monte Fenera, in NW Italy, is a protected area of 3378 ha (Fig. 1), included in the Sites of Community Importance list (code IT1120003). Evidence of the use of the caves since the Riss-Würm interglacial was documented by the description of fossils of at least 9 species of bats (Berto *et al.*, 2016). Our sample sites are comprised in the municipalities of Borgosesia, Valduggia and Grignasco. Most of the protected area is a carbonate-built hill that is home to 81 karstic caves at an altitude of 600-800 m a.s.l. The environment surrounding the caves' entrance is mainly composed of woods, with some cultivated areas and small villages from 2-3 km henceforth.

Winter areas

We inspected the four largest caves, namely:

- Bondaccia (Italian cave registry code PI2505); WGS84 45.7114438°N, 8.3118285°E, 690 m a.s.l., 500 m-long.
- Ciota Ciara (code PI2507); WGS84 45.7108912°N, 8.3112443°E, 675 m a.s.l., 202 m-long.
- Ciutarun (code PI2506); WGS84 45.710138°N, 8.3102257°E, 655 m a.s.l., 66 m-long.
- Arenarie (code PI2509); WGS84 45.711919°N, 8.3145207°E, 770 m a.s.l., 3 km-long.

Summer-autumn areas

We inspected the environment surrounding the Monte Fenera cave area, grouping the observations among four habitat categories:

- cave entrance: the sampling covers the space (about 20-30 m) in front of the entrance of three large caves, Bondaccia, Ciota Ciara and Ciutarun.
- woods: five sampling points. The woods are dominated by chestnut which is accompanied by other species such as ash, English oak, sessile oak, Turkey oak, birch, maple, aspen, wild cherry, willow, and mountain ash; black locust has invaded the southern hills, while in the humid valleys and along the streams there is alder and black poplar. To the north, on the cooler slopes, beech is abundant, while to the south, on rocky walls and calcareous soils, there is manna ash, a typical essence of Mediterranean environment to which butcher's broom and juniper also belong, which are found in abundance. Natural conifers (Scots pine) are present sporadically while the most common are those of planting, preferred by man for their rapid growth (such as strobe pine). Among typical shrubs in the woods are hazelnut, dogwood, elder, hawthorn, barberry and privet.
- agricultural mosaic with light: two sampling points. Open spaces with grass vegetation and orchards, with small buildings and the presence of light coming from the houses.
- dark agricultural mosaic: two sampling points. Open spaces with grass vegetation and orchards, with no buildings nor light.

Areas with incidental observations

We utilized some occasional data collected by speleologists in smaller caves:

- La Caudrola PI2735: WGS84 45.7045478°N, 8.3101146°E, 515 m a.s.l.
- Buco delle Marmitte della cava Antoniotti PI2550: WGS84 45.7006103°N, 8.312411°E, 402 m a.s.l.
- Buco delle Radici PI2540: WGS84 45.7116969°N, 8.3225534°E, 804 m a.s.l.
- Bell'ingresso PI2539: WGS84 45.7104921°N, 8.3242896°E, 754 m a.s.l.

Other data were collected from citizens reporting dead specimens, and in a single event placing mist nets at the Bondaccia cave entrance in October 2012.

Field surveys

Winter census

Hibernating individuals were monitored through standardized counts carried out in their underground roosts between the last five days of December and the first ten days of February (Battersby, 2010). Hibernation site surveys were repeated once every year at each site in order to minimize disturbance. Bats were counted directly or with binoculars along all human accessible tracts in caves, or with the aid of images in case of aggregated groups that required the use of photography or video cameras (Toffoli & Calvini, 2021).

Summer-autumn census

In our study area, the year-round reproductive life cycle of bats typically starts with the autumnal swarming-mating period, followed in spring and early-summer by a female-lactating period. In late summer, both adults and recently born bats can be observed foraging. In this study we inspected the environment utilized by foraging bats from early July to early October, thus encompassing both the lactating period and the periods when young bats start to forage independently, with some overlapping with the mating period.

Bat activity was investigated using AudioMoth bat detectors (Hill *et al.*, 2019), with their microphones placed 1.5 m above the ground. Real time recordings were made all-night-long at a sampling frequency of 256 kHz. We started bat acoustic sampling half an hour after sunset until half an hour before sunrise. This time interval includes the main peaks of activity for most insectivorous bats of the temperate zone (Erkert, 1982). Recordings were made on nights without precipitation, with wind strength below 6 m/s and temperature above 10 °C at sunset, to avoid measuring low bat activity due to weather factors. All recordings were then divided in segments of 5 sec duration using the Kaleidoscope program 3.1.1 (Wildlife Acoustics, Maynard, Massachusetts, USA).
To describe activity levels, the number of passes per hour of each different species and acoustic groups was used (Barataud, 2016). A pass is defined as a recording consisting of at least one echolocation signal (call) up to a total length of 5 sec (Thomas & West, 1989; Barataud, 2016). In the results, we reported the average number of bat passes / hour in order to take into account the difference in the number of sampled sites and the recording time in the different habitat categories.

Data analysis

Winter census

Population trend was analyzed with the TRIM software (Pannekoek & Van Strien, 2001) through the package "Rtrim" 2.0.6 within the R statistical environment (Bogaart *et al*., 2016). TRIM implements log-linear Poisson regression models to analyse time series of count data (Gregory *et al.*, 2005), and was developed for the analysis of time series of counts with missing values (Pannekoek & van Strien, 2001). It has been utilized to analyze trends in hibernating bats (Uhrin *et al*., 2010; Van der Meij *et al*., 2015; Toffoli & Calvini, 2019).

Summer-autumn census

Species identification was obtained by analyzing each bat call sequence following the methodological approach provided by Barataud (2016) and comparing the data with those published by Russo & Jones (2002). The identification method is based on the signal shape (FM: frequency modulation; QCF: quasi constant frequency), the energy peak distribution and on the measurement of the following parameters: start frequency (SF), end frequency (EF), bandwidth (BW), frequency of maximum energy (FME) in kHz, duration (D) and inter-pulse interval in ms (IPI). To reduce the time spent examining sonograms, in a first step we utilized the Sonochiro® 3.3.2 software (Biotope Society, France) for automatic identification of echolocation signals of European bats (Azam *et al*., 2015; Charbonnier *et al*., 2016). This software identifies signals in a recorded sequence and classifies them at a homogeneous species group level according to characteristics of signals. The software attributes a reliability index from 0 to 10 for each identification. In a second step, we controlled manually all sequences with a score lesser than 5, as indicated by Toffoli & Rughetti (2017), to attribute sequences to one species or one acoustic group according to the characters reported in Barataud (2016), Russo & Jones (1999) and Middleton *et al.* (2014).
Signals belonging to the genus *Myotis*, excluding those classified at a specific level, were incorporated into the two groups *Myotis* LF (*Myotis myotis* and *Myotis blythii*) and *Myotis* HF (all other species), due to the difficulties encountered in identifying them at species level (Russo & Jones, 2002). Due to a lack of firm discrimination criteria for bioacoustic distinction between *Plecotus auritus*, *Plecotus austriacus* and *Plecotus macrobullaris*, we attributed all signals of the genus to the *Plecotus* sp. group.
With regard to *Pipistrellus kuhlii* and *Pipistrellus nathusii*, whose identification is difficult (Barataud, 2016), only sequences with a score equal to or greater than 9 were attributed to one of the two species. All others were controlled manually and only those with the characteristic social calls were identified at species level (Russo & Jones, 1999; Middleton *et al*., 2014), while the remnants were referred to the acoustic group *Pipistrellus kuhlii/nathusii*.
Sequences with FME signals from 20 to 30 kHz and the presence of characteristic alternate FM/QCF and QCF signals of different FME were associated to the *Nyctalus* genus (Waters & Jones, 1995). Sequences of primarily FM/QCF signals either longer than 17 ms and with end frequency between 21 and 24 kHz, or shorter than 5 ms and with EF between 29 and 32 kHz were associated to *Eptesicus serotinus* (Barataud, 2016). Concerning the identification of European Free-tailed Bat *Tadarida teniotis*, in order to exclude *Nyctalus lasiopterus* and the social calls of *Nyctalus leisleri*, reference was made to the guidelines of Barataud (2016). In particular, in order to exclude *Nyctalus lasiopterus*, sequences without alternating structure between QFC and FM signals and with a maximum energy frequency between 9 and 13 kHz have been attributed to *Tadarida teniotis*, while as regard the exclusion with the social calls of *Nyctalus leisleri*, reference was made to the initial form of the characteristic signal of the social calls of *Nyctalus leisleri*.

RESULTS

Winter census

The winter censuses in the four largest caves of Fenera along the 17-years monitoring period confirmed the cumulated presence of 534 *Rhinolophus ferrumequinum* (501 at Bondaccia, 18 at Ciota Ciara, 11 at Arenarie, 4 at Ciutarun), while other species where much less abundant: 3 large *Myotis* (*M. myotis/blytii* at Bondaccia), 1 small *Myotis* (*M. alcatoe/brandtii/mystacinus* at Bondaccia), 1 *Myotis crypticus* and 1 *Pipistrellus* sp. at Ciota Ciara.

The trend of observed and estimated number of wintering *Rhinolophus ferrumequinum* is reported in Table 1, with a clear and steady increase of census individuals.
The TRIM population analysis model shows a significant overall gradient of 1.22 (Wald test; $p < 0.001$), which corresponds to an average annual increase of 22 % over the period considered (Fig. 2).

Table 1. Number of hibernating *Rhinolophus ferrumequinum* bats in Fenera caves, and trends (TRIM index, TRIM estimates and standard errors) along 17 winters. The dashes refer to winters when no inspections were done.

Winter	Observed individuals	TRIM index	Estimated individuals	SE
2004/2005	5	1	5	5
2005/2006	-	1.2192	6	3
2006/2007	-	1.4987	8	3
2007/2008	-	1.8424	10	4
2008/2009	-	2.2648	12	4
2009/2010	-	2.7841	15	4
2010/2011	-	3.4225	18	5
2011/2012	24	4.3923	24	10
2012/2013	-	5.2987	27	6
2013/2014	36	6.9713	36	12
2014/2015	-	7.6223	41	6
2015/2016	-	9.6077	51	6
2016/2017	63	11.9867	63	16
2017/2018	65	13.0798	69	18
2018/2019	92	17.5044	92	20
2019/2020	101	20.2935	107	22
2020/2021	148	29.4829	155	25

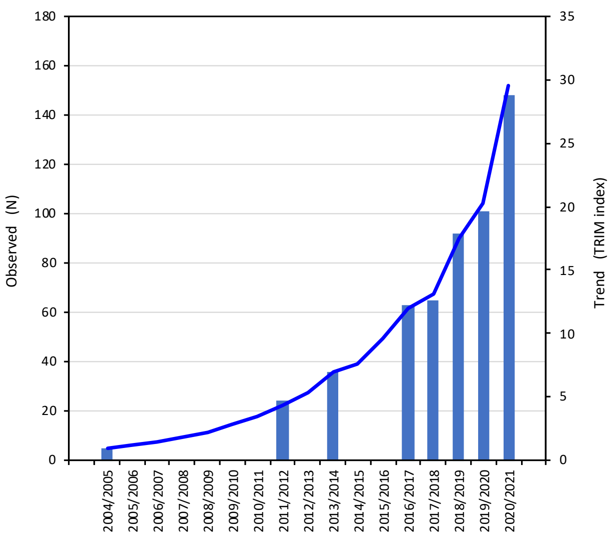

Fig. 2. Variation of wintering *Rhinolophus ferrumequinum* bats in the period 2004/2005 - 2020/2021.

Summer-autumn census
Data on the activity of bat species, or bat acoustic groups, in the four different habitats surrounding the cave area are reported in Table 2. The most utilized habitat was the agricultural mosaic with light, which showed a 2-3-fold activity level respect to the other habitats. These high activity levels were mainly due to the most ubiquitous species of the genus *Pipistrellus* and of the acoustic group *Eptesicus/Nyctalus/Vespertilio*.
The habitats utilized by the higher number of species were the woods and the cave entrances. The most active species in all four habitat categories was *Pipistrellus pipistrellus*, while the acoustic group *kuhlii/nathusii* was particularly active in the agricultural mosaic with light, as well as *Nyctalus leisleri*.
Several species where rarely observed (Table 2), except for the high activity of *Rhinolophus ferrumequinum* recorded in the area surrounding the cave entrances.

Incidental observations
A checklist of all species observed in the Fenera cave area is reported in Table 3. The list includes the incidental observations reported below:
- *Tadarida teniotis*: recorded with a bat-detector at the entrance of Ciota Ciara cave on 6 October 2012;
- *Rhinolophus ferrumequinum*: a single individual roosting in a small building at Colma (summer 2018);
- *Pipistrellus pipistrellus*: 1 ind. recorded with a bat-detector at the entrance of Ciota Ciara cave (summer 2018); 1 ind. found dead at Colma (summer 2018); 2 ind. roosting in the roof of a house at Maretti Valduggia (summer 2020);
- *Plecotus austriacus*: 1 ind. found dead at Zuccaro Valduggia on 17 June 2021;
- *Plecotus auritus*: 2 ind. captured by mist net at the entrance of Ciota Ciara cave on 6 October 2012.

DISCUSSION

Our study reports the presence of 18 species of bats found wintering or flying in the area surrounding cave entrances in a karstic area with several caves in NW Italy. In the winter period, according to visual inspections of caves, hibernating bats were almost exclusively *Rhinolophus ferrumequinum*. This species is scored as Near Threatened in the European Mammal Assessment (Temple & Terry, 2007). Situations of local extinction of the species have been reported in parts of England, Holland, Israel. Negative demographic trends were recorded in Austria, Belgium, Germany, Bulgaria, France, and Switzerland, followed only recently by a positive trend (Van der Meij *et al.*, 2015). In Great Britain, numerical decreases have been documented in the early 60s and 80s, while current populations seem stable. In Italy the species was probably abundant in the last century (Gulino & Dal Piaz, 1939), but surveys

Table 2. Activity of bat species or bat acoustic groups in four environments surrounding the cave entrances in Fenera's area. Activity is reported as mean number of bat passes per hour.

Species/Acoustic groups	**Woods**	**Cave entrances**	**Dark agricultural mosaic**	**Agricultural mosaic with light**
Barbastella barbastellus	0.03	0	0	0.03
Eptesicus serotinus	0.38	0.12	0.47	5.32
Eptesicus/Nyctalus/Vespertilio	0.45	0.10	0.21	17.97
Hypsugo savii	1.13	2.90	0.44	0.35
Myotis crypticus	0.03	0.38	0.02	0
Myotis daubentonii	0.10	0	0	0
Myotis emarginatus	0	0.18	0	0
Myotis HF	0.86	1.26	0.54	0.85
Myotis LF *myotis/blythii*	0.04	0.03	0.36	0.65
Nyctalus leisleri	0.05	0.03	3.44	23.82
Nyctalus noctula	0	0.05	0	0
Pipistrellus kuhlii	1.85	0.86	1.38	2.88
Pipistrellus kuhlii/nathusii	6.38	2.90	5.56	50.94
Pipistrellus pipistrellus	60.55	35.43	38.12	51.09
Pipistrellus pygmaeus	0.15	0.49	0	0
Plecotus sp.	0.01	0.02	0.48	6.38
Rhinolophus ferrumequinum	0.01	17.48	0.03	0
Tadarida teniotis	0	0	0.03	0
TOTAL BAT ACTIVITY (passes/hour)	**72.0**	**62.2**	**51.1**	**160.3**
Sampling effort (hours)	**79.5**	**115.5**	**66.5**	**34.0**

carried out recently show a notable rarefaction compared to the past (Agnelli *et al.*, 2004). The population is in decline due to the loss of foraging habitats, due to the intensification of agriculture and the use of pesticides, as well as to the reduction of adequate refuge sites. Large colonies are very rare (usually few individuals per colony, rarely more than 100 individuals). Our data highlight the importance of the Fenera caves as one of the most important wintering areas in NW Italy for this species. The large numerical increase that occurred during the last decade, as well as the high number of individuals counted in the last five years, are probably related both to the recent trend observed in NW Italy (Toffoli & Calvini, 2021) and to the conservation actions which have been put in place since the 1998, with the assembly of closed metal gates that prevent access to unauthorized tourists but allow the entry and exit of bats.

Our study highlights the interest of the Fenera as a foraging area utilized by *Rhinolophus ferrumequinum* during the summer and autumn seasons too. In NW Italy, during the 90s, observations of reproductive sites utilized in spring or summer were completely lacking (Sindaco *et al.*, 1992). To avoid possible disturbance of maternity roosts, we did not explore the caves in the summer period, but the presence of the species with high levels of activity is a good indication of reproduction in the area, considering that females usually have a much reduced home range during the lactating period (Jeon *et al.*, 2018). Alongside, there may have been a substantial contribution to the high activity rate of the area due to the presence of commuting males (Downs *et al.*, 2016) who possibly had daytime rest areas nearby. The presence of woods is also important for *Rhinolophus ferrumequinum*, the preferred foraging habitats of which are broad-leaved woodland interspersed with open spaces (Duvergé & Jones, 1994; Ransome & Hutson, 2000; Flanders & Jones, 2009).

Our study provides the first data of foraging activity in a NW Italy karstic area. The most utilized habitat was the agricultural mosaic with light, which showed a high activity level mainly due to the most ubiquitous species of the genus *Pipistrellus* and of the acoustic group *Eptesicus/Nyctalus/Vespertilio*. Besides, the habitats utilized by the higher number of species were the woods and the cave entrances. However, we underline that a fine comparison of the number of species in the different habitats would have required a much more extensive sampling, with the calculation of accumulation curves (Moreno & Halffter,

Table 3. Checklist of bats species observed in the caves of Fenera.
*: data reported in Pascutto & Balestrieri (2001) for the period 1993-1999, with supplementation from Lana *et al.* (2021) and the authors up to 2021.

Sites	*Tadarida teniotis*	*Rhinolophus ferrumequinum*	*Rhinolophus hipposideros*	*Barbastella barbastellus*	*Eptesicus serotinus*	*Hypsugo savii*	*M. alcathoe/brandtii/mystacinus*	*Myotis daubentonii*	*Myotis emarginatus*	*Myotis myotis/blythii*	*Myotis crypticus*	*Nyctalus leisleri*	*Nyctalus noctula*	*Pipistrellus kuhlii*	*Pipistrellus pipistrellus*	*Pipistrellus pygmaeus*	*Plecotus auritus*	*Plecotus austriacus*
Years 1993-1999 (2021)*																		
Bondaccia Cave		x	*							*							*	
Ciutarun Cave		x																
Ciota Ciara Cave		x	*															
Arenarie Cave		x																
Small caves		x	x							x								
Small building																		*
Years 2004-2020 (winter)																		
Bondaccia Cave		x					x			x								
Ciutarun Cave		x																
Ciota Ciara Cave		x									x							
Arenarie Cave		x																
Year 2020 (acoustic)																		
Cave entrances		x			x	x			x	x	x	x	x	x	x	x		
Woods		x		x	x	x		x		x	x	x		x	x	x		
Agricultural mosaic with light				x	x	x				x		x		x	x			
Dark agricultural mosaic	x	x			x	x				x	x	x		x	x			

2001; Skalak *et al.,* 2012). These habitats were utilized by some rare species, i.e. *Barbastella barbastellus* (Toffoli & Cucco, 2020) and all species of the genus *Myotis.* Our results are in line with the observations of Zukal & Řehák (2006) in the Moravian karst, with the exception of the high bat activity noted over Moravian ponds, a habitat that was not present in the Fenera area. The importance of karstic areas outside of the hibernating period has been underlined in several tropical zones (Struebig *et al.,* 2009; Racey & Furey, 2014), but has been little studied in temperate areas. Our results underline the importance of protecting the cave underground system as well as the habitats surrounding cave entrance for an effective conservation of bat biodiversity.

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