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GIANNA DONDINI ⁽¹⁾, SIMONE VERGARI ⁽¹⁾

“POGGIO ALLA GUARDIA” MINE (PISTOIA PROVINCE): A KEY HYPOGEUM SITE FOR BATS CONSERVATION IN TUSCANY

Abstract - G. DONDINI, S. VERGARI, *Poggio alla Guardia” Mine (Pistoia Province): a key hypogeaum site for bats conservation in Tuscany.*

Data from a research carried out from 2015 to 2022 on bats in the hypogea of Poggio alla Guardia (Pieve a Nievole, Pistoia) are presented. The aim of this work was to collect data during wintering on the populations of the various species present in order to understand the phenology and numerical trend. Overall, 5 species have been identified that use the site differently during the various seasons. In particular, *Rhinolophus ferrumequinum* Schreber, 1774 and *Miniopterus schreibersii* Kuhl, 1817 exploit the hypogeaum both for wintering and for reproduction, while *Rhinolophus hipposideros* Bechstein, 1800 is present exclusively for the winter. As regards *Rhinolophus euryale* Blasius, 1853 and *Plecotus austriacus* J. Fischer, 1829, they are present in small numbers and in a discontinuous manner, not allowing precise indications on the trends. With the data collected, a significantly negative trend was highlighted for *R. ferrumequinum* while for *R. hipposideros* and *M. schreibersii* a stability in the size of the wintering colonies. A close relationship was highlighted between the use of the various sectors of the hypogea and the recorded temperature. The site is of fundamental importance for the conservation of bats in northern Tuscany due to the combination of its extension and microclimatic diversity. Measures that limit disturbance are necessary, especially during particularly sensitive periods in the biological cycle of bats.

Key words - hypogea, bats, conservation, Tuscany, Italy

Riassunto - G. DONDINI, S. VERGARI, *La miniera di “Poggio alla Guardia” (Provincia di Pistoia): un ipogeo chiave per la conservazione dei pipistrelli in Toscana.*

Vengono presentati i dati di una ricerca effettuata dal 2015 al 2022 sui pipistrelli presenti negli ipogei di Poggio alla Guardia (Pieve a Nievole, Pistoia). Scopo del presente lavoro è stato quello di raccogliere dati durante lo svernamento sulle popolazioni delle varie specie presenti al fine di comprendere la fenologia e l'andamento numerico. Complessivamente sono state identificate 5 specie che utilizzano il sito in maniera differenziata durante le varie stagioni. In particolare *Rhinolophus ferrumequinum* Schreber, 1774 e *Miniopterus schreibersii* Kuhl, 1817 sfruttano l'ipogeo sia per lo svernamento che per la riproduzione. Mentre *Rhinolophus hipposideros* Bechstein, 1800 è presente esclusivamente per lo svernamento. Per quanto riguarda *Rhinolophus euryale* Blasius, 1853 e *Plecotus austriacus* J. Fischer, 1829 sono presenti con piccoli numeri e in maniera discontinua non permettendo di avere precise indicazioni sugli andamenti. I dati raccolti evidenziano un andamento significativamente negativo per quanto riguarda *R. ferrumequinum* mentre per *R. hipposideros* e *M. schreibersii* una stabilità nella consistenza delle colonie svernanti. È stata evidenziata una stretta relazione tra utilizzo dei vari settori degli ipogei e la temperatura registrata. Il sito riveste una fondamentale importanza per la conservazione dei pipistrelli nella Toscana settentrionale per la combinazione

tra la sua estensione e diversità microclimatica. Sono necessarie misure che ne limitino il disturbo soprattutto durante i periodi particolarmente sensibili nel ciclo biologico dei pipistrelli.

Parole chiave - ipogei, pipistrelli, conservazione, Toscana, Italia

INTRODUZIONE

For northern Tuscany, there are few published data on underground shelters and their use by bats (Agnelli *et al.*, 1999). More consistent data are available only for the province of Prato, where more recently specific studies have been conducted (Agnelli *et al.*, 2009). Hypogea represent important refuges for bats all over the world (Furey & Racey, 2016; Mickleburgh *et al.*, 2002; McCracken, 1989). At least 28 out of the over 40 European bat species find refuge in hypogea during hibernation, and some of them throughout the year (Furey & Racey, 2016; Dietz *et al.*, 2009). The use of hypogea offers a remarkable advantage in terms of thermal stability and high humidity, which protect bats against excessive water loss (Avila-Flores & Medellín, 2004; Gunn, 2003). Moreover, utilizing caves serves to mitigate the risk of predation, as bats frequently congregate in subterranean sections that are challenging to access (Furey & Racey, 2016). Thermoregulation is also facilitated in these congregations (Kunz, 1982). The main disadvantage of hypogea is their relative scarcity, and often remoteness from favourable hunting areas. Data relating to a multi-year research aimed at creating a list of species that use the hypogea of Poggio alla Guardia (Pieve a Nievole Municipality, Pistoia Province) are presented here. Furthermore, numerical trends of the species are reported, and possible correlations with the microclimate of the various sectors of the site are established. The lack of data on bats from this important site significantly limits the possibilities of implementing concrete conservation and territorial management actions, bats being excellent bioindicators and keystone species (Russo & Jones, 2015; Jones *et al.*, 2009). So the data reported here can be used to promote environmental protection actions and implement correct forms of management.

⁽¹⁾ MuNAP - Museo Naturalistico Archeologico Appennino Pistoiese, via Orange, 51028 Gavinana, Pistoia
Corresponding author: Gianna Dondini (dondinigianna@gmail.com)

MATERIALS AND METHODS

Site study

The hypogea of Poggio alla Guardia are situated in an old disused mine in the southern part of the province of Pistoia (Municipality of Pieve a Nievole, Northern Tuscany) on a hill characterized by rocks referable to the M. Morello Formation, made up of carbonate Flysch, marly limestone and marl (Cantini *et al.*, 2001; Trevisan *et al.*, 1971). The mine is divided into two branches that are very close but not communicating with each other, with two respective entrances. The first entrance is located at an altitude of 140 m a.s.l., the second at an altitude of 130 m a.s.l. The climate of the area, based on the Köppen classification (Belda *et al.*, 2014), is warm temperate with dry summer (Cs). The area to the south and south-west of the hypogeum is particularly urbanized; to the south-east the small Montalbano ridge and an important wetland area (Marshes of Fucecchio) occur, whereas the Apennine area, characterized by a forest cover of oaks (*Quercus cerris* L.), chestnuts (*Castanea sativa* Mill.) and beeches (*Fagus sylvatica* L.) occur to the north.

The mine of Poggio alla Guardia is particularly complex and its length exceeds 2000 m. The two branches of the mine both develop on three levels connected by stairs carved into the rock.

Data collection

Surveys were carried out from 2015 to 2022 with an interruption in 2021 due to the lockdown linked to the COVID-19 epidemic outbreak. Data for winter 2014 are available for *Rhinolophus hipposideros* Bechstein, 1800. For each study year, the two quarries were completely inspected once in the summer (June-July) and once in the winter (December-January). Bat counting was performed for *Miniopterus schreibersii* Kuhl, 1817 and *Rhinolophus ferrumequinum* Schreber, 1774 through a visual count of single individuals and a photographic count for clusters. Once a cluster of bats was photographed, each individual was marked with a red dot on the photo for a more precise numerical estimate and subsequently adding the two values to have an estimate of the 'minimum number present'. For *Rhinolophus hipposideros*, *Rhinolophus euryale* Blasius, 1853 and *Plecotus austriacus* J. Fischer, 1829, counting was performed exclusively by sight (Agnelli *et al.*, 2004).

For the thermal profile of the hypogea, in 2021 four data logger sensors U23-001 from HOBO-ONSET were used. They measured the temperature once a day for a whole year in four sectors of the hypogea. Three sensors were located in branch 1 on the 'first level', 'second level' and 'third level'. A fourth sensor was placed in branch 2 at its deepest point of the first

level, named 'hibernating'. Relative humidity was measured until mid-November, then the humidity sensors stopped working. For the analysis of trends in bat population, only data from the winter censuses are used.

Data analysis

PAST 4.03 software was used for statistical analyses. A one-way ANOVA followed by a Tukey's pairwise post hoc test was used to determine whether there were any significant differences in temperature inside the mine. To evaluate the numerical trend of wintering bats, a linear regression was performed (Ordinary Least Squares method).

RESULTS

Five species of bats have been identified in the hypogea of Poggio alla Guardia (Tab. 1). For *M. schreibersii* ($t = 2.181$, $r^2 = 0.4875$, $P = \text{ns}$, Fig. 1) and *R. hipposideros* ($t = 1.62$, $r^2 = 0.3058$, $P = \text{ns}$, Fig. 2), no significant numerical change was highlighted while a significant decrease was highlighted in *R. ferrumequinum* ($t = 3.04$, $r^2 = 0.6496$, $P = 0.028$, Fig. 3). For *R. euryale* and *P. austriacus* there are not enough data to evaluate their status and any numerical changes during the study period.

The yearly average temperatures in the four sectors of the hypogeum were significantly different (Fig. 4). In particular, in branch 1 the first level had an average temperature (\pm SD) of 13.2 °C (\pm 2.8; $n = 353$), the second level 15.6 °C (\pm 2.9; $n = 353$), and the third level 18.3 °C (\pm 2.6; $n = 353$). In the second branch the average temperature is 10.1 °C (\pm 2.0; $n = 353$). There is a significant difference in the four surveyed sectors (ANOVA, $df = 3$, $F = 622.4$ $P < 0.01$). Tukey test showed a significant difference in average temperature between all four levels.

A relationship between average temperatures and the use of the mine by the various species was highlighted. In particular, *R. ferrumequinum* and *M. schreibersii* massively used the second branch of the mine for hibernation, where the temperature was always particularly low and below 10 °C during the winter months (Fig. 4 and Tab. 2). The same two species used the second level and third level of branch 1 for reproduction where temperatures were particularly high and suitable for this phase of the biological cycle (Fig. 4 and Tab. 2). Relative humidity always remained between 80% and 100% in all sectors of the hypogea. The temperature and humidity of the hypogea are generally constant (Gunn, 2003) and we can assume that the measurements taken in a year are representative of the microclimate.

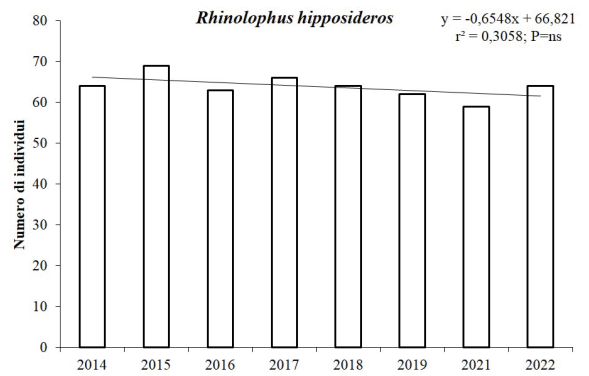
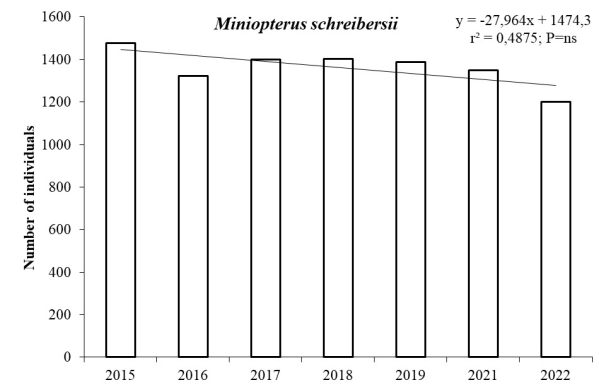


Figure 1. Number of wintering individuals of *Miniopterus schreibersii*.

Figure 2. Number of wintering individuals of *Rhinolophus hipposideros*.

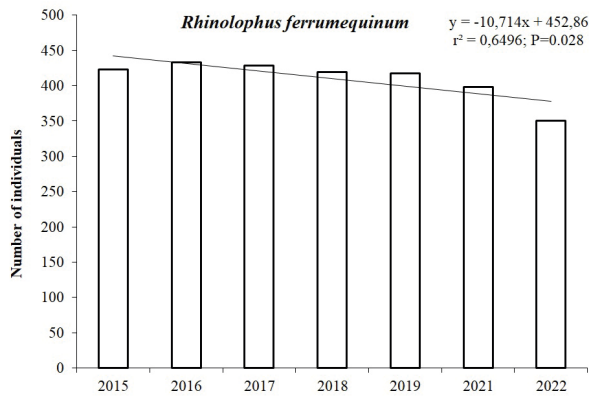


Table 1. Species reported in the hypogea of Poggio alla Guardia. R= reproduction; W= wintering; NK= not known.

Species	Cave use	Habitat Directive
<i>Rhinolophus ferrumequinum</i>	R, W	Annex II
<i>Rhinolophus euryale</i>	W	Annex II
<i>Rhinolophus hipposideros</i>	W	Annex II
<i>Miniopterus schreibersii</i>	R, W	Annex II
<i>Plecotus austriacus</i>	NK	Annex IV

Figure 3. Number of wintering individuals of *Rhinolophus ferrumequinum*.

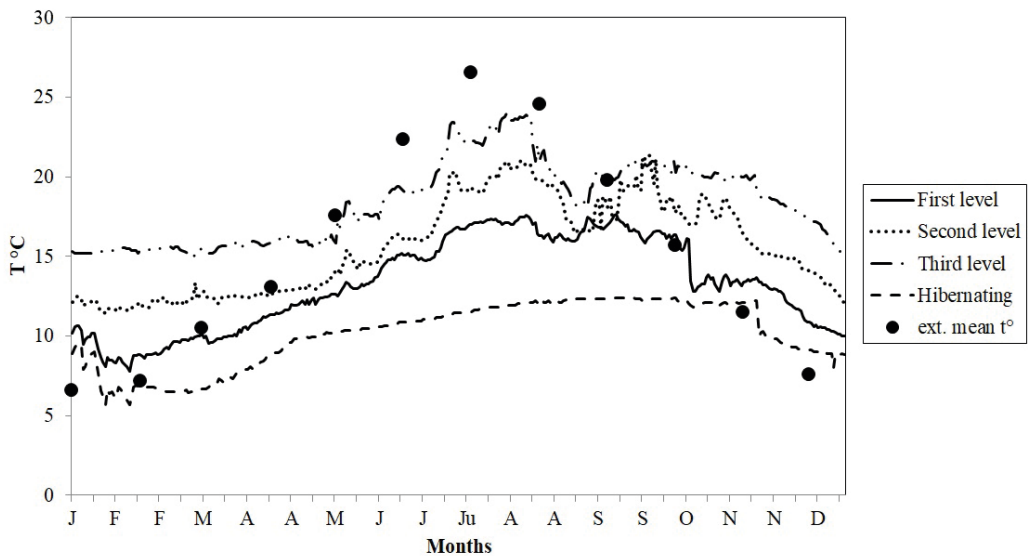


Figure 4. Temperature variation in four sectors of the two branches of the mine, compared with the Pontelungo meteorological station (Tuscany Region code: TOS01004837, 67 m a.s.l.). The “hibernating” sector is in branch 2 while the other are in branch 1 and distributed over three different level.

Table 2. Use of the various sectors of the mine during the year by the five species of bats present. There is a close relationship between the average temperatures recorded in the various sectors and the activities related to the biological cycle. B1= branch 1 of the mine; B2= branch 2 of the mine.

	J	F	M	A	M	J	Ju	A	S	O	N	D
<i>R.e.</i>	Wintering B1 FIRST LEVEL				Not present					Wintering B1 FIRST LEVEL		
<i>R.f.</i>	Wintering B2 HIBERNATING		Reproduction B1 SECOND LEVEL					Swarming?		Wintering B2 HIBERNATING		
<i>R.b.</i>	Wintering B1 FIRST LEVEL		Not present					Wintering B1 ground floor				
<i>M.s.</i>	Wintering B2 HIBERNATING		Reproduction B1 SECOND LEVEL					Swarming?		Wintering B2 HIBERNATING		
<i>P.a.</i>	Not present					FIRST LEVEL B2		Not present				

DISCUSSION

The hypogean site of Poggio alla Guardia is characterized by a diversity of temperatures, thus favouring not only the reproduction by some species of bats, but also their wintering. Hibernation is an excellent adaptation to overcome periods with low temperatures and low prey availability and is a characteristic of the biological cycle of many species of microbats in the temperate climatic zone (Ransome, 1990). During hibernation, bat body temperature falls to within 1-2°C of ambient temperature and metabolic processes slow down, thereby reducing energy requirements (Humphries *et al.*, 2003). In general, wintering takes place in a temperature range above freezing and below 10°C (Boyles *et al.*, 2007; Webb *et al.*, 1995; Nagel & Nagel, 1991). It is particularly important to note that the thermal profile in branch 2 of the Poggio alla Guardia mine during the winter months is conducive to the wintering of bats. This is due to the denser cold air accumulating in the deepest part of the mine. In the first branch of the mine due to the presence of several floors that rise upwards, we find sectors with high temperatures throughout the year and favourable for the formation of nurseries (Bauerová & Zima, 1988; Anthony *et al.*, 1981). This microclimatic diversity is a key factor for the Poggio alla Guardia site to represent a strategic refuge for bat conservation at least at a regional level. Considering that in the hypogean of Poggio alla Guardia we find also individuals of *R. ferrumequinum* coming from the Tuscan coast, over 50 km away (Dondini *et al.*, 2009), preserving this refuge is pivotal. During the study we observed a significant decrease in *Rhinolophus ferrumequinum* in terms of wintering individuals. The available data are inadequate to correlate this decrease to disturbances within the mine or to other unknown factors. We consider the Poggio alla Guardia hypogean as an extremely important site for the conservation of bats and highlight the need to develop a correct strategy for its conservation, maintaining its usability for bats and limiting its disturbance as much as possible (Mitchell-Jones *et al.*, 2007).

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