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The bats of the Rio Martino Cave, North West Italy (Mammalia Chiroptera)

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ABSTRACT We present the results of a monitoring of the hibernating bats (Mammalia Chiroptera) in the Rio Martino Cave (North West Italy) and a survey of swarming activity. In total, 13 species of cave-dwelling bats were detected. During winter monitoring, 12 species of bats were recorded. The most abundant bat was *Barbastella barbastellus* (Schreber, 1774) which represents 91.5% of the total hibernating bats documented in the cave, and its number has increased significantly, reaching a maximum of 410 bats in the winter of 2013–2014. A significant positive trend in population increase was also observed for *Myotis emarginatus* (E. Geoffroy Saint-Hilaire, 1806). Between 2009 and 2010, a total of 354 bats belonging to 9 species were captured, thus confirming swarming activity for *Myotis emarginatus* and the presence of *M. bechsteinii* (Kuhl, 1817). The results confirm the importance of the Rio Martino Cave for the conservation of cave-dwelling bats in the Italian western Alps.

KEY WORDS Mammalia; Chiroptera; monitoring, hibernating; swarming; Italian Alps.

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INTRODUCTION

The importance of the Rio Martino Cave for the bats has long been known, but due to the lack of studies on bats, the historical information available in this regard is fragmented. In the early 1960s, the cave was a target location for a ringing activity carried out by the Bats Ringing Center of the Italian Speleological Society. Unfortunately it is difficult, if not impossible, to use the chiropterological information gathered during this experience, since the overall reports published are incomplete and, in part, contradictory. However, from the available sources (Dinale, 1965; Martinotti, 1968), it is clear which individuals in the winter of 1960–61 were captured in the cave and ringed or collected as specimens for museum destinations. These individuals belonged to at least 4 species of bats: *Rhinolophus hipposideros* (Bechstein, 1800) (at least one specimen), *Myotis blythii* Tomes, 1857 and/or *M. myotis* Borkhausen, 1797 (at least 4 specimens), *Myotis emarginatus* (E. Geoffroy Saint-Hilaire, 1806) (at least 2 specimens), and *Barbastella barbastellus* (Schreber, 1774) (at least 24 specimens: 11 ringed on 8.XII.1960, of which 3 were recaptured on 6.I.1961, when a further 11 bats were ringed and 2 specimens were collected for the Doria Museum of Genoa, Italy). Subsequently, chiropterological information for the cavity was no longer available until the 1990s.

Since the winter of 1991–1992, the cave is regularly monitored to assess the number and the trend

of wintering bats and is subject to specific surveys with the aim of verifying the bats' annual presence and their use of the cave as a swarming site. Winter monitoring was partially financed in the 2004–2009 period by the Biodiversity and Natural Area Office of Piedmont Region (Debernardi et al., 2010).

The aim of this work is to provide a summary of current knowledge about the bats in the cave of Rio Martino based on records collected during twenty-eight years of survey and bat monitoring, evaluating their richness, population trends, and use of this habitat.

MATERIAL AND METHODS

Study area

The cave of Rio Martino (Crissolo, Cuneo, North West Italy, 44°42.017'N – 7°8.881'E) is the most important natural underground cavity of the Italian Cottian Alps with an entrance 1,530 meters above sea level, a development of 2,905 metres and a difference level of 191 metres (+174, -17) (AGSP 2010). The entrance is located on the right side of the Po Valley (Fig. 1), in an area characterized by mixed deciduous forests and *Larix decidua* Mill. The cave is designated as Special Areas of Conservation (SAC) IT1160037 and is currently managed by the Natural Park of Monviso.

The average temperature in the cave it is approximately 4° C with a humidity close to 100%. Daily temperature fluctuations are very slight, of the order of 0.02° C, and there is continuous unidirectional ventilation with a speed between 5 and 48 meters per minute (Badino, 2008).

The troglobiotic fauna is rather poor and typical of cold caves and is at a high altitude with the presence of Diplopoda, such as *Crossoma semipes* Strasser, 1958, and predatory Opilions, such as *Ischyropsalis* cfr. *alpinula* Martens, 1978 (Lana, 2001).

Bat sampling

The methods used to survey and monitor the bats in the Rio Martino cave refer to direct counts

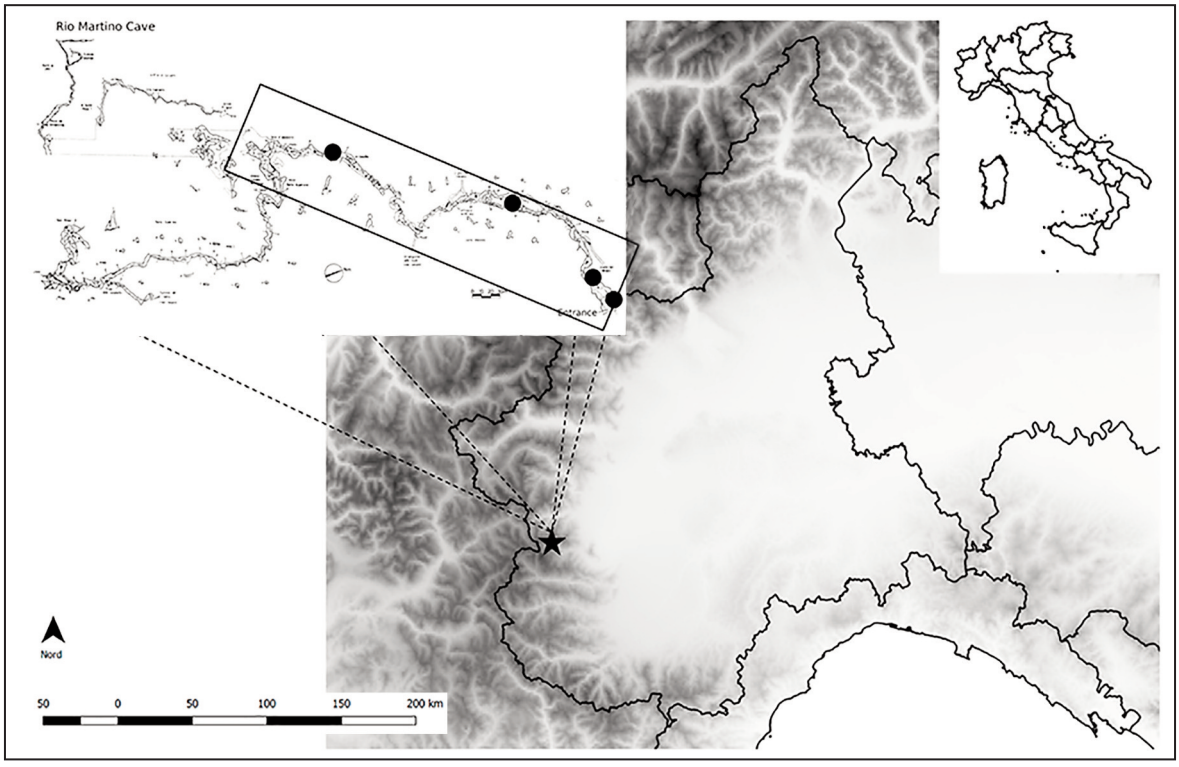


Figure 1. Study area. Star: localization of the cave; rectangle: part of the cave being monitored; black dots: temperature/humidity data logger position.

of hibernating individuals and to captures with mist-nets and harp-traps in late summer-early autumn. During the hibernation phase, individuals were counted directly on site, or in the case of large and aggregated groups, with photographic images taken from the site. In order to minimize the disturbance, a single annual survey beginning from winter 1991–1992 to winter 2018–2019 was generally carried out on a central date of the lethargy period (from 15 December to 15 February, preferably between the end of December and mid-January). In some winters, two counts were carried out and, in such cases, the count with the largest number of bats was used. From June 2009 to May 2010, in addition to monitoring hibernating bats, monthly visits were carried out to verify the annual phenology. All counts were always made in the same portion of the cave (from the entrance to the "Pissai" cascade) to allow comparison.

During the visits, all bats were identified based on differences in morphology at the species level, when possible (Nyssen, 2015), in some cases with the help of 10x42 binoculars. In cases where the species could not be identified due to intrinsic difficulties, the genus or species group was used instead.

The number of hibernating bats during the last fourteen winters has been correlated with the average temperature of the month preceding the count in order to verify correlations between meteorological variables. The data relating to the temperatures have been obtained from the weather stations of ARPA Piemonte (Regional Agency for Environmental Protection) located at Crissolo (Cuneo) available on https://www.arpa.piemonte.it/rischinaturali/accesso-ai-data/annali_meteoidrologici/annals hydro-meteorological/bank-data-meteorologica.html.

In 2009 and 2010, captures were also carried out to verify the use of the cave as a swarming site. The captures were made during the nights of 16–17 August, 18–19 September 2009 and 15–16 September 2010, using a harp trap at the entrance of the cave which was active from sunset until 04:00. All individuals captured were identified to species level, and the sex, reproductive status, and age according

Figure 2. Cluster of 205 *Barbastella barbastellus* in winter 2012–2013 (cave of Rio Martino, Crissolo, Cuneo, North West Italy).

Hibernation season	*Rhinolophus hipposideros*	*Barbastella barbastellus*	*Eptesicus serotinus*	*Myotis alcathoe/brandtii/mystacinus*	*Myotis blythii*	*Myotis myotis* vel *blythii*	*Myotis daubentonii*	*Myotis emarginatus*	*Myotis nattereri*	*Pipistrellus* sp	*Plecotus* sp	*Miniopterus schreibersii*	**Total bats**	**Number of species**
1991/1992	1	7	1		1	6		2		1			**19**	**7**
1992/1993		10			1	5	1	2					**19**	**5**
1993/1994		11			1	8		1					**21**	**4**
1994/1995		10			2	9		1					**22**	**4**
1995/1996		10			1	4		1	1		1		**16**	**4**
1996/1997		17			3	6		1			1		**28**	**5**
1997/1998		18	1		2	7		2	1				**29**	**5**
1998/1999		16	1		2	5	1	2					**27**	**6**
1999/2000		21			2	6		2	1				**30**	**4**
2000/2001		41			2	12		4					**59**	**4**
2001/2002		44			2	2	1	5					**54**	**5**
2002/2003		76			1	11		2					**90**	**4**
2003/2004		82			2	5		1					**90**	**4**
2004/2005		129			5	19	1	2					**156**	**5**
2005/2006		178	1		8	2	4	9					**202**	**6**
2006/2007		183		2	7	3	3	6					**204**	**6**
2007/2008		272			5	2		3					**282**	**4**
2008/2009		295			6	2	1	3					**307**	**5**
2009/2010		375	1		10	2	1	1					**390**	**6**
2010/2011		380			4	3		4					**380**	**4**
2011/2012		367			1	9	3	2			1		**383**	**6**
2012/2013		399	1		3	8		6					**417**	**5**
2013/2014		410	1		4	3		5			1		**424**	**6**
2014/2015		380	1		3	1		5			1		**391**	**6**
2015/2016	1	170	1	1	7	10		3					**193**	**7**
2016/2017		293	1		9	4		7					**314**	**5**
2017/2018		193			10	7		19		1		1	**231**	**6**
2018/2019		195			4	9	1	18				1	**228**	**6**

Table 1. Number of hibernating bats in Rio Martino Cave.

to the ossification of the phalanges was recorded (Dietz & Von Helversen, 2004; Brunet-Rossini & Wilkinson, 2009; Haarsma, 2008). Bats were subsequently released after being marked on their toes with a non-toxic paint to check for possible recaptures.

The captures were made under permission of the Italian Ministry for the Environment, Land and Sea (DPN/2008/0001053; DPN/2010/0011879).

Temperature and humidity parameters of the cave were monitored from July 2009 to September 2011, through the placement of 4 data loggers Testo 174T (Fig. 1) set to record data every hour.

RESULTS

Surveys and monitoring, conducted over the years, allowed to detect the presence of 13 bats species in the cave.

During the winter monitoring, presence of 12 species of bats was detected with a maximum of 7 species and a minimum of 4 for the hibernation period (Table 1). Four species were found on every winter: *Barbastella barbastellus*, *Myotis blythii*, *M. emarginatus*, and *M. myotis* vel *blythii*. One species was detected for ten winters, *Eptesicus serotinus* (Schreber, 1774), one was detected for nine winters, *Myotis daubentonii* (Kuhl, 1817), while the others were recorded for no more than five winters.

The most abundant bat was *Barbastella barbastellus* which represents 91.5% of the total hibernating bats counted in the cave (mean ± SD = 162 ± 151), and the number of individuals has increased significantly ($Rs = 0.90$, $p<0.001$) reaching a maximum of 410 bats during the winter of 2013–2014 and then decreasing (Table 1). This species is observed in hibernation as isolated individuals or as large clusters (> 200 bats) (Fig. 2). The annual variations in the number of bats belonging to this species are inversely correlated with the average temperature values of the month before the count (Fig. 3), and this correlation is significant. (Wilcoxon test $Z = -3.29$, $p<0.001$). As for the other species observed every year, there is a significant positive trend ($Rs = 0.66$, $p<0.001$) only for *Myotis emarginatus*, while for large *Myotis* (*M. blythii* and *M. myotis*) there is no defined trend.

The location of the different species of bats in the cave is influenced by the characteristics of the air, including temperature and humidity. *Barbastella barbastellus* is present from the entrance to the first half of the monitored part of the cave. In this sector, the temperatures show an average value in the period from 15 December–15 February of 0.7 °C (± SD 1.3) with an average humidity of 84.2% (± SD 18.7), with wide fluctuations (temperature min. -1.5 max 3.5, humidity min. 42.3% max 99.9%). In this part of the cave, the species *Eptesicus serotinus*, *Pipistrellus* sp., *Plecotus* sp., and *Miniopterus schreibersi* (Kuhl, 1817) were also observed.

The large *Myotis*, *Rhinolophus hipposideros*, *Myotis emarginatus*, and the other small *Myotis* are observed in the deepest part of the cave where the temperature is on average higher and constant (mean ± SD = 6.2 ± 0.5, min. 5.7 max 7.1) with a high mean humidity of 95.0% (± SD 3.5).

Monthly counts made between 2009 and 2010 showed the presence of bats in every month of the year with the exception of August (Table 2). Three species (*Barbastella barbastellus*, *Eptesicus serotinus*, and *Myotis daubentonii*) were detected only during the hibernation period in late autumn and early spring. As for *Barbastella barbastellus*, their presence was detected from October to March, with a peak in January and February. Only the large *Myotis* (*M. myotis* and *M. blythii*) were observed every month, with the exception of August, while *Myotis emarginatus* was also detected in the spring during the months of April and May, as well as September and in the winter.

The captures made in 2009 and 2010 detected a total of 354 bats belonging to 9 species (Table 3). Most of the captured bats were male, while only 57 were females (16.1%). The majority of males showed evident reproductive traits, with swollen testicles (68.2% of captured males). The most abundant species of all the three capture sessions was *Myotis emarginatus* (71.5% of the total), followed by *Myotis daubentonii* (14.7%) and *Barbastella barbastellus* (8.5%). The hourly trend of the captures showed a peak between 22:00 and 01:00 with variations between the three sessions (Fig. 4).

DISCUSSION

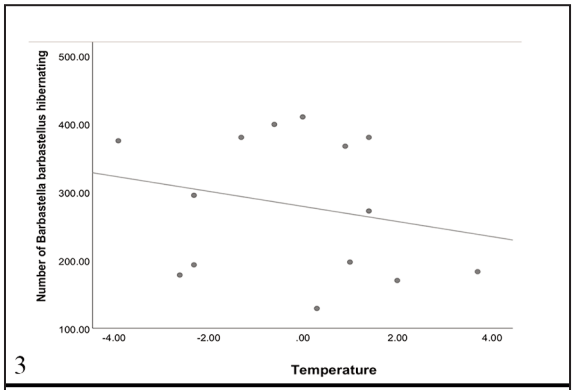
The results confirm the importance of the Rio Martino cave for the bats. The 12 species detected

in hibernation, a number which could be higher as unidentified species could hide within these groups, represent the highest number of species for a winter site in Italy (GIRC, 2004). Of particular importance is the high number of *Barbastella barbastellus* in hibernation. The Rio Martino cave is the most important hibernation site for this species in Italy, since there are only six wintering sites currently known of which only one has no more than 100 individuals (GIRC, 2004). The long-term trend observed for this species corresponds to what was observed during the winter in other European countries (e.g., Poland, Slovakia) where stable or increasing trends were observed (Lesiński et al., 2005, 2011; Uhrin et al., 2010) or more generally significant positive trends with an average annual increase of 4% in Europe are occurring (Van der Meij et al., 2015). The decrease observed from the winter of 2014–2015 is difficult to explain. It is however evident that the number of *Barbastella barbastellus* observed in the last fourteen winters is correlated with the average temperature of the month preceding the count, and the last three winters have been characterized by fairly high temperatures compared to the average (https://www.arpa.piemonte.it/rischi-naturali/accesso-ai-data/annali_meteoidrologici/annals hydro-meteorological/bank-data-meteorologica.html). Also, for *Myotis emarginatus* the hibernating trend corresponds to what was generally observed in Europe (Van der Meij et al., 2015). For the other species, it is not possible to make any consideration about the trends observed due to the limited number of individuals observed.

Of some interest are the observations of *Rhinolophus hipposideros* and *Miniopterus scheribersii* hibernating in the cave. *Rhinolophus hipposideros* was recorded as present in the Rio Martino cave during the 1960s (Martinotti, 1968; Sindaco et al., 1992) and observed in hibernation in the winter of 1991–1992. Subsequently, the species was no longer detected until the winter of 2015–2016, probably as a consequence of the increase in the number of hibernating bats observed at other sites in the province of Cuneo (Toffoli, 2005). *Miniopterus schreibersi* is a rare species in Piedmont, and to date, the only locality where the species has been recorded is a gypsum mine in the province of Cuneo at 210 meters above sea level where arguably the species has reproduced once (Boano & Curletti, 1974) and where it is currently present with a single

	Barbastella barbastellus	*Myotis myotis* vel *blythii*	*Myotis blythii*	*Myotis daubentonii*	*Myotis emarginatus*	*Eptesicus serotinus*	**Total bats**	**Number of species**
June		1	1				**2**	**2**
July		1					**1**	**1**
August							**0**	**0**
September		4	1				**5**	**2**
October	15	2	2		1		**20**	**4**
November	35	2	2				**39**	**3**
December	193	1	9		1	1	**205**	**5**
January	375	2	10	1	1	1	**390**	**6**
February	380	4	8	1	3	1	**397**	**6**
March	38	1	3		2	1	**45**	**5**
April		1			1		**2**	**2**
May		1			1		**2**	**2**

Table 2. Number of bats counted monthly from 2009 to May 2010.



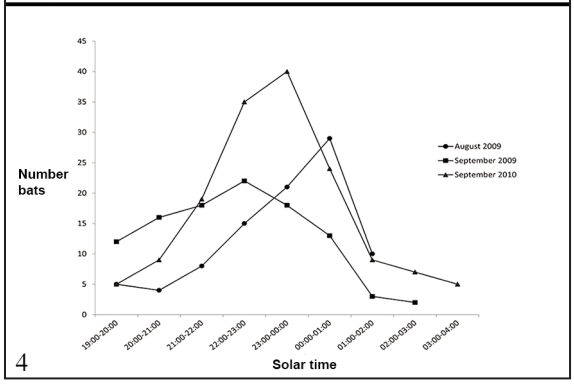


Figure 3. Correlation between the number of *Barbastella barbastellus* hibernating and the average external temperature of the cave in the month preceding the count. Figure 4. Hourly variations in the number of bats captured at the entrance to the cave.

	Barbastella barbastellus	*Myotis bechsteinii*	*Myotis blythii*	*Myotis daubentonii*	*Myotis emarginatus*	*Myotis mystacinus*	*Myotis myotis*	*Myotis nattereri*	*Plecotus auritus*	**Total**
August 2009	7		2	5	77			1		**92**
September 2009	8	1	3	30	56	1		5		**104**
September 2010	15		1	17	120		1	3	1	**158**
Total	**30**	**1**	**6**	**52**	**253**	**1**	**1**	**9**	**1**	**354**

Table 3. Number of bats captured at the entrance of the cave.

specimen in autumn and winter (Debernardi et al., 2010; Toffoli, unpublished data). The winter observations in the Rio Martino cave represents a second site of presence for the species in Piedmont and is one of the highest altitude observations in Italy. The species is reported in Italy up to 1,050 meters above sea level on the central Apennines (Lanza, 2012), although there are reports of its presence up to about 1,500 meters in Austria (Spitzenberger, 1981) and up to 1,630 meters in the French Alps (Drousie & Cosson, 2016).

As for the characteristics of temperature and humidity where the different species of bats have been detected in the cave, these coincide with the ranges available in bibliography (Bogdanowicz & Urbańczyk, 1983; Daan & Wichers, 1968; Webb et al., 1996) with values comprised between -3.0° and 6.5° for the most cold-tolerant species (e.g., *Barbastella barbastellus*) and between 5.0° and 10.5 for those mostly related to higher temperatures (e.g., *Myotis emarginatus*) (Webb et al., 1996).

The correlation between the number of hibernating *Barbastella barbastellus* and the average temperatures of the month preceding the count can be partly explained by the greater flight activity and the possible movements between the different roosts. The external temperature can, in fact, stimulate the activity of the bats in hibernation making them change the roost and shelter, in particular for the individuals who hibernate closer to the entrances of the caves where the microclimatic variations are greater (Daan, 1973). This may suggest movements of *Barbastella barbastellus* wintering in roosts exposed to climatic variations towards the cave of Rio Martino during periods of lowering temperatures.

The presence of bats through all months of the year increases the conservation value of the cave. The absence of bats observed in daytime rest during the month of August is probably not real, but linked to the difficulty of observing the few individuals often hidden in deep crevices of the rock. The presence of bats in this month is, in fact, confirmed by their capture at the cave entrance.

The activity detected in the late summer trough captures, the hourly variations, and the composition in age and sex classes of the captured bats are consistent with those expected for swarming sites (Parsons et al., 2003, 2003a; Šuba et al., 2008). The number of species and individuals detected is high and is greater than any other known swarming sites in Piedmont (Debernardi et al., 2010; Toffoli & Culasso, 2010). Bats detected during swarming correspond to the hibernating species with the exception of *Myotis bechsteinii* (Kuhl, 1817) which was not detected during winter counts. The absence of this species in winter can be explained by the behaviour of hiding

in deep cavities making observation difficult (Groupe Chiroptères de la LPO Rhone-Alpes, 2014).

The results confirm the importance of the Rio Martino Cave for the conservation of bats, with at least 13 species detected, of which 12 were observed in hibernation. In particular, the cave has great importance at an Italian level for the wintering of *Barbastella barbastellus*, but it is also an important swarming site for all the other species living in the Italian western Alps. Last but not least, the cave plays an important role for the diurnal shelter of individuals during the spring and summer.

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