ORIGINAL ARTICLE

Long-term monitoring of temperature effect on the population dynamics of the only overwintering colony of *Myotis blythii* in the Iberian Peninsula

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ABSTRACT

Hibernation is a critical period for the survival of bats in temperate regions. In Southern Europe in particular, the hibernation of some species is hampered by the scarcity of winter roosts with temperatures low enough to reach a deep hibernation state, which involves physiological processes other than daily torpor. In this paper we present the results of the study on various aspects of the only known wintering colony of the lesser mouse-eared bat (*Myotis blythii*) in Spain. Located in a cave in the Sierra de las Nieves National Park (Málaga), the colony has been monitored for ten years by means of photographic censuses and climatic data recording. As a result, it has been determined that at least 93.75% of the colony belongs to *Myotis blythii*. The characteristic whitish tuft between the ears, present in a variable proportion in European *M. blythii*, was in at least 85% of the individuals. The population fluctuates during the period of roost occupation, from September to May, reaching a maximum in March 2010 when up to 1517 bats were counted. In this month the temperature of the roost reached its minimum: 4.02 °C ± 1·18 (mean ± SD). The population was distributed in groups of different sizes with a mean of 35.50 individuals ± 76.54 (mean ± SD), ranging from solitary individuals (1.0% of the total observed) to clusters of more than 600 bats. Overall, we found that temperature had an inverse relationship on the cluster size, while water vapor pressure deficit had a direct relationship. Specifically, the best fit was for the mean temperature and mean water vapor pressure deficit for the previous 15 days. Climate change, to which the Mediterranean region is especially vulnerable, may alter the conditions of this refuge, posing a threat to this unique colony.

INTRODUCTION

Energy conservation in winter is a limiting factor for mammals, due to the high cost of maintaining homeothermy in adverse environmental conditions with scarce food availability, especially for bats as they have a limited fat storage capacity (Speakman & Rowland 1999, Geiser 2004, Jonasson & Willis 2012, Stawski et al. 2014). This is overcome by hibernation, a temporary heterothermic state in which there is a reduction in metabolism below basal level and, consequently, body temperature, and allows conserving energy and water, key to survival and reproduction (Hock 1951, Lyman et al. 1982, Ransome 1990, Ben-Hamo et al. 2013, Lazzeroni et al. 2018, Haase et al. 2019). Hibernation requires specific environmental conditions that many bat species in temperate regions encounter in caves (Davis 1970, Jepsen 1970, Tuttle & Stevenson 1978), where two environmental factors are particularly relevant: i) temperature, as it affects the metabolic rate by determining energy expenditure (Hock 1951, Wojciechowski et al. 2007)

and ii) water vapor pressure, as a measure of atmospheric humidity, for its close relationship with evaporative water loss (Studier 1970, Thomas & Cloutier 1992, Thomas & Geiser 1997, Boyles et al. 2017). In addition, hibernation is influenced by other factors, such as age, individual energy reserve, potential availability of food or behavior, such as clustering (Daan 1973, Clawson et al. 1980, Koteja et al. 2001, Kokurewicz 2004, Boyles et al. 2007, Boyles & Brack 2009). During the hibernation process, bats are periodically aroused by raising their body temperature to euthermic levels (Lyman et al. 1982, Czenze et al. 2013). These arousal episodes represent the greatest energetic cost of the hibernation period (Thomas et al. 1990, Dunbar & Tomasi 2006) and they occur for intrinsic and extrinsic factors, such as an increase in ambient temperature (Ransome 1990). How increasing temperatures linked to climate change will affect hibernation is poorly understood (Festa et al. 2022). Some studies suggest climate change may affect aspects such as the length of the hibernation period (Stawski & Geiser 2012), total energy requirements, biogeographical

distribution (Humphries et al. 2002), population dynamics (Festa et al. 2022), synchrony with insect phenology (Meyer et al. 2016) or exposure to diseases, i.e. white-nose syndrome (McClure et al. 2022). Stawski & Geiser (2012) propose that it is possible that the heterothermic capacity of bats to use torpor efficiently in conditions of thermal variations may favor their adaptation to climate change.

The lesser mouse-eared bat, *Myotis blythii* (Tomes, 1857), is a gregarious mainly cave-dwelling species, with a wide South Palearctic and Oriental distribution; ranging from the European Mediterranean, to Southern Russia, through the Middle East and Central Asia, to Northeastern China (Strelkov 1972, Simmons 2005, Ruedi 2020). In the Iberian Peninsula, it is widely distributed except in Northwestern and Central Spain where it is less abundant (Nogueras & Garrido 2007). Although it has a preference for altitudes below 600 m a.s.l. (Ibáñez 1998), breeding colonies have been observed up to 1350 m (Paz et al. 2015) and hibernating up to 2100 m in Sierra Nevada, Southern Spain (Nogueras & Garrido 2007). In Central Europe, the maximum distance between summer and winter roosts reach 150 km, most commonly at 50 km or less (Topál 1956, Gaisler et al. 2003). The maximum range recorded has taken place in Spain, with 488 km (Paz et al. 1986, Hutterer et al. 2005, Ruedi 2020). Flemming & Eby (2003) classify this species as a regional migrant. They feed on a wide variety of prey belonging to the orders Orthoptera, Coleoptera, Lepidoptera and Diptera (Arlettaz 1996, Arlettaz et al. 1997a, Benda et al. 2006, Whitaker & Karataş 2009, Benda et al. 2010, Machado 2017).

In Europe, lesser mouse-eared bats generally hibernate in groups of large to medium size or even singly. They hibernate in crevices, small interstices or freely in caves or other underground roosts with temperatures below 12 °C (Gaisler 1970, Schober & Grimmberger 1989, Benda et al. 2008, Dietz & Kiefer 2017), located at higher altitudes than the breeding roosts (Uhrin et al. 2008). More specifically, in Portugal hibernation mainly occurs in caves and mines, occupied by isolated individuals or small groups, although there are also shelters with several hundred (Palmeirim & Rodrigues 1992, ICFN 2014). In Spain, according to the scarce data available, hibernation occurs in underground shelters, natural or artificial (Ibáñez 1998). No large colonies are known so far. From censuses in breeding colonies, the estimated total population of *M. blythii* for the Iberian Peninsula is approximately 22000 individuals (Queiroz et al. 2005, Nogueras & Garrido 2007), of which 8300 are found in the Andalusia region, Southern Spain (Ibáñez et al. 2005). The population trend of *M. blythii* is decreasing, both globally and locally for the Iberian Peninsula (Rodrigues et al. 2003, Nogueras & Garrido 2007, Juste & Paunović 2016). In Andalusia, although the historical trend has been clearly downward, population trend is currently uncertain (Nogueras & Garrido 2007). Population estimates for this species remain uncertain due to possible confusion with its twin species, the greater mouse-eared bat, *Myotis myotis* (Borkhausen, 1797) (Nogueras & Garrido 2007, Consejería de Medio Ambiente 2011, ICFN 2014).

This study, carried out in the main winter roost for bats in the Sierra de las Nieves National Park, aims to: 1) identify the species present, 2) analyze the population dynamics of the deep hibernating colony of *M. blythii*, the only one known in the Iberian Peninsula, 3) evaluate the influence of climatic factors on aggregation patterns, and 4) provide information to National Park managers for use in the conservation of the colony.

MATERIAL AND METHODS

Study Site

The research was carried out in a natural cave located at 1745 m a.s.l. in the Sierra de las Nieves National Park (Málaga province, Southern Spain) (Fig. 1) in Mesozoic limestone (Olmo et al. 1990). The park authorities have information on its location, which is not detailed here for conservation reasons. The general appearance of the surrounding area is mainly rocky, subjected to erosive processes of the karstic type, with grassland vegetation, high mountain scrub and very sparsely dense gall oak grove (*Quercus faginea* Lam.) The roost, schematically, consists of a shaft 57 m deep with a total surface area of about 430 m^2 (Muñoz 1982). Its main chamber, where the bats hibernate, is located at the bottom and its 348 m^2 floor plan is approximately triangular in shape, with a sloping roof between 4 and 11 m high and some standing water during winter and spring. The walls and roof are predominantly smooth, due to erosion that has affected the paleo-speleothems (Delannoy 1998). In addition, other caves in the area have been surveyed to check for the existence of other colonies. Specifically, in the Sierra de las Nieves National Park, ten caves were explored within a radius of 5 km around the known roosting cave (range - altitude: 730 - 1686 m a.s.l., length: 50 - 18657 m, depth: 54 - 803 m, temperature: 4.0 - 6.4 °C). In the Natural Park of Sierra Tejeda (Canillas de Aceituno, province of Malaga), 89 km from our cave, another chasm with similar characteristics was explored (altitude: 2061 m asl, length: 29.6 m, depth: 45.1 m, temperature: 3.2 °C).

Fig. 1 - Sierra de las Nieves National Park location map, where the colony is located. https://commons.wikimedia.org/wiki/File:Comarca_de_la_Sierra_de_las_Nieves.svg

Bat surveys

A total of 41 censuses were carried out in the cave across ten years: between April 2008 and March 2018. In each of census, during daylight hours for about 45 minutes, two people collected samples of dead specimens and took photographs of the colony with a digital camera (Olympus SP-500UZ, Olympus Imaging Corp. Tokyo, Japan) and flash (Vivitar 728AFNi, Vivitar Corp. Santa Mónica CA USA), without handling or marking the animals. A goal throughout the information collection process was to minimize the number of visits and to avoid any disturbance beyond what was necessary for accurate data collection (Speakman et al. 1991, Thomas 1995). Due to the scarcity of hidden areas in the cave, we surmise that the vast majority of the colony was counted in each sampling. For three nights it was checked if there was activity of entry or exit of bats (22/09/2012, 20/12/2013 and 29/03/2018). Subsequently, the numbers of individuals and clusters within the photographs were counted using image editing software (Adobe Photoshop cs. V. 8.0.1. 1990-2003 Adobe Systems Incorporated). We define a cluster as the set of two or more bats between which there was the possibility of heat exchange by physical contact. We also calculated the proportion of solitary individuals in relation to the total number of bats present.

Species identification was carried out on morphological characteristics, both from collected specimens and photographs. According to their characteristics they were grouped into: i) osteological material of cranio-mandibular bones collected in the cave (n = 65) using at least one of the following morphometric features: greatest skull length, zygomatic breadth, condylobasal length (CBL), length maxillary tooth row (CM^3) or mandibular length (Arlettaz et al. 1997b, Paz & Benzal 1989). The age of the osteological material used has not been determined. Measurements were made with a Vernier caliper (Mitutoyo, ± 0.02 mm accuracy) under a stereo microscope and the osteological material was deposited in the Department of Animal Biology of the University of Málaga; ii) dead individuals were also collected from the cave floor (n = 9), and at least two of the following morphometric features were used to determine species identification depending on their state of decomposition: forearm length, condylobasal length, maxillary tooth row (canines to molars, CM^3) length, mandibular length, ear length, Arlettaz et al. (1997b) external characters discriminant function (Z = 0.433 x forearm length + 3.709 x ear length - 114.887) and the presence or absence of a whitish spot between the ears (Paz & Benzal 1989, Palmeirim 1990, Dietz & von Helversen 2004). Finally, iii) the determination of the live individuals photographed was carried out by identifying the general morphological characteristics of the species, with particular attention to the presence or absence of a whitish spot on the forehead, between the ears, and a dark spot on the tip of the tragus (Arlettaz et al. 1991, Dietz & von Helversen 2004). The analysis was performed on a total of 2550 bats photographed in the censuses; individuals in which the presence or absence of these characters was not clearly observed were excluded from the count.

Climatic data

Climatic data were recorded for outside and inside the roost to assess the possible influence of climate on roost occupancy period and clustering. Ambient outside temperature was estimated from the Ronda Meteorological Station data (S.A.I.H. Hidrosur 2019) (altitude: 770 m a.s.l., distance to the cave: 15.5 km) between September 2010 to March 2018, applying the lapse rate correction factor of 0.65 °C / 100 m (Mokhov & Akperov 2006, Wallace & Hobbs 2006), i.e., in our case 6.34 °C was subtracted from the temperature recorded at the meteorological station.

Air temperature and humidity inside the cave were measured every hour for 1992 days between November 2011 to March 2018 using a data logger (Hobo Pro V2 U23-001, Onset Computer Corp., Bourne USA) (accuracy: ± 0.2 °C, ± 2.5 - 4% relative humidity) placed two meters high from the floor in the main room of the cave. Water vapor pressure (WVP) (hPa) has been used as an absolute measure of the humidity of the air inside the cave (Anderson 1936, Kurta 2014) and, based on Fick's law of diffusion (Withers & Cooper 2014), we consider water vapor pressure deficit (WVPD) as a proxy of possible water loss from each animal (Procter & Studier 1970, Baudinette 1972, Christian 1978, Webb et al. 1995). The WVPD values were obtained by subtracting the WVP value (corresponding to the ambient relative humidity) from the saturated water vapor pressure (SWVP) (corresponding to the ambient temperature). The Tetens equation modified by Brice & Hall (2019) was used. The daily oscillation was established by subtracting the maximum and minimum daily temperature and WVP. There were periods of no data due to failures with the data logger. For validation, occasional measurements were carried out with an instantaneous reading thermometer Pronamic in/out digital thermo (accuracy: ± 2 °C) (Pronamic ApS., Skjern Denmark) and thermal images were taken with Fluke®TIR1 thermal camera (accuracy: ± 2 °C) (Fluke Corporation, Everett USA).

Statistical analysis

The trend of outdoor and indoor temperature and DAPV (mean, minimum and maximum) during the study period were obtained by fitting a simple regression model. We also used a linear simple regression to evaluate the influence of these climatic variables for different time periods (1, 2, 3, 5, 10 and 15 days) on colony size. Both statistical analyses were carried out using package Statgraphics Centurion XVI, Version 16.1.03 (StatPoint Technologies, Inc. 1982-2010). On the other hand, the influence of the indoor climatic variables (mean, minimum, and maximum), for these same periods, on the clustering (number of bats in each cluster) was analyzed using generalized linear mixed models (GLMMs) with 'glmmTMB' package with R for Windows v. 4.1.1. Fifteen models were generated (three models for each time period) including the temperature and the WVPD variables and their interaction as predictors. Models were built with a negative binomial distribution for overdispersed count data and a log-link (Zuur et al. 2009). Year was included in all models as a normally distributed random effect. If the interaction term was not statistically significant (P < 0.05), it was dropped from each model. Final models were visually validated (Zuur & Ieno 2016) and tested for overdispersion and zero-inflation with 'DHARMa' package. The optimal model displaying the greatest parsimony and goodness of

fit was selected based Akaike information criterion (AIC) and graphically represented using 'jtools' package.

RESULTS

Species identification

The cranio-mandibular bones found belonged to *M. blythii* in 93.75% of those collected (n = 60) and the remaining 6.25% (n = 4) belonged to *M. myotis* (Table 1). All the dead individuals collected were identified as *M. blythii* (Table 2). For the live animals we observed, dark coloration of the tragus tip, exclusive of *M. myotis*, was present in 3 of the 167 bats where this feature was visible (1.80%). On the other hand, 2080 of the 2430 individuals observed in the successive censuses (85.60%) had a whitish spot between the ears, usual but no always present in *M. blythii* (Table 3). It should be noted that the total number observed is greater than the size of the colony, so that on occasions the same bats was probably observed repeatedly on different dates. We found no evidence of any other bat species using the roost for hibernation.

Occupancy period and population fluctuation

The colony occupies the cave mainly from September to May (Table 4). During these months, bats remain in a state of hibernation, as evidenced by the absolute absence of droppings or food remains, no flight at the cave entrance during the night, low body and ambient temperatures (body thermal image: 0.1 - 0.8 °C; rock wall thermal image: -0.7 °C; surrounding air temperature: 2.2 °C) (Fig. 2) and stability in clusters compositions after one week (09/03/2014 - 16/03/2014) (Pearson correlation coefficient = 0.997; P < 0.001) (Fig. 3). Inside the main room, the colony occupied walls and ceilings no less than two meters above the floor. In this same room, on two occasions, footprints and droppings of *Martes foina* (Erxleben, 1777) were found, but without evidence of predation on bats.

Fig. 2 – Thermal image of *M. blythii* taken in the cave in December 2016. Wall temperature -0.7 °C (30.7 °F); body temperature between 0.1 °C (32.1 °F) and 0.8 °C (33.4 °F). Surrounding air temperature: 2.2 °C (instantaneous reading thermometer).

Roost occupancy increases gradually between September and March, the month in which it reaches its peak (Table 4). Subsequently the population decreases rapidly during April and May and remains absent or in small numbers from June to August. This pattern has remained constant throughout the years of study. In relation to variations in population size over the years, it has been observed that in the period 2016-17 and 2017-18 the number of individuals has decreased with respect to previous years. For example, the annual mean in December in the 2016-18 period was 268.50 individuals, while in previous years it was 850.14 individuals (Table 4).

The colony has shown a clear tendency to form clusters of different sizes. Solitary individuals, in absolute numbers, were scarce, representing only 1.0% of the total number of bats observed. However, in relative terms, they accounted for a significant percentage of the population between the months of May and October (mean 22.9%), with a peak in October 2016 of 15 solitary out of 22 individuals in total (68.2%).

Fig. 3 – Cluster comparison one week apart. Total clusters 09/03/2014: n = 26; 43.85 bats ± 126.36 (mean ± SD); Total clusters 16/03/2014: n = 26; 43.92 bats ± 129.43 (mean ± SD). The figure shows in detail one of the clusters observed.

Table 1 - Mean and standard deviation (SD) of different cranial measurements taken from the skeletal remains belonging to *M. blythii* and *M. myotis* collected in the cave. GS: Greatest skull, ZW: Zygomatic width, CB: condylobasal, CM³: maxillary tooth row, M: mandible. Number of variables measured in each case depended on their state of preservation. Due to their small number, the standard deviation of *M. myotis* has not been calculated.

	Myotis blythii			*Myotis myotis*		
	n	Mean (± SD)	Range	n	Mean	Range
GS	21	21.34 (± 0.34)	20.84-21.98	2	23.61	23.53-23.68
ZW	17	14.01 (± 0.31)	13.29-14.47	0	-	-
CB	24	20.19 (± 0.53)	18.68-21.02	2	22.29	22.10-22.48
CM³	41	8.39 (± 0.21)	8.02-8.78	2	9.67	9.49-9.84
M	22	16.44 (± 0.35)	15.87-17.30	2	17.87	17.85-17.88
Total (%)	60 (93.75%)			4 (6.25%)		

Table 2 - Length values (mm) of the variables examined in nine carcasses. FA: forearm, CB: condylobasal, CM³: maxillary tooth row, M: mandible. Z: discriminant function values for variables from Arlettaz et al. 1997b. All these values are in accordance with those established for *M. blythii*.

	1	2	3	4	5	6	7	8	9
FA	57.8		59.7	60.9	49.9				
Ear			20.7	21.4					
CB		20.60				20.65	20.70	19.85	20.05
CM³	8.80	8.50	8.50	8.40	8.10	8.70	8.65	8.25	8.25
M						16.70	16.55	16.20	
Z		-4.79	-3.67						

Bats and outdoor temperature

During the ten years of monitoring, the estimated interannual outdoor temperature has shown an almost stable trend for the mean, maximum and minimum (mean: $y = -0.0039x + 11.139$; maximum: $y = -0.0032x + 15.43$; minimum: $y = -0.0039x + 7.3929$). Overall, the annual temperature outside the cave was 11.01 ± 7.82 (mean ± SD). July represented the warmest month (22.51 °C mean ± 2.58 SD) and February the coldest (2.30 °C mean ± 2.80 SD). The daily oscillation of the outside temperature was 8.12 °C ± 1.98 (mean ± SD), reaching the minimum in December (5.84 °C mean ± 2.46 SD) and the maximum in July (11.14 °C mean ± 1.74 SD) (Fig. 4). Temperature outside the cave (daily, maximum and minimum means) during the largest colony size period (November to April) exerts a significant inverse effect, which fits an exponential model, on the population dynamics in all of the time periods evaluated (1, 2, 3, 5, 10 and 15 days). The maximum values of the coefficient of determination (R^2) are reached for the mean maximum outdoor temperature of the last fortnight (Colony size = exp. ($8.12005 - 0.235502*$ Mean maximum temperature last fortnight); Correlation coefficient: -0.88; R^2: 77.91%; ANOVA P-value < 0.001; F = 59.96; df = 18).

Bats and indoor climate: Effect on population fluctuation

Indoor temperature data showed a slight downward trend throughout the study period, although this is likely due to the lack of readings in the warmer months (Fig. 5) (mean: $y = -0.0047x + 5.0414$; maximum: $y = -0.0035x + 5.1044$; minimum: $y = -0.0057x + 4.9754$). Overall, the mean annual temperature inside the cave was 4.87 °C ± 1.40 SD, with October as the month with the highest temperature (6.66 °C mean ± 0.24 SD) and February the coldest (3.05 °C mean ± 0.99 SD). The daily mean temperature during November to April, when the colony size was the largest, was 4.02 °C compared to 5.97 °C for the rest of the year. Very stable, the daily temperature oscillation throughout the entire study period was 0.21 °C ± 0.21 (mean ± SD). There were only 25 of the 1992 days-where the oscillation range was recorded between 1 - 2 °C, the rest were less than 1 °C. June was the month with the lowest range (0.10 °C) and March the highest (0.33 °C).

During the hibernation period, there is a statistically significant inverse relationship between number of individuals in the colony and indoor temperature that fits an exponential model. That is, the largest wintering population sizes were reached in the coldest periods. The maximum values of the coefficient of determination (R^2) are reached for the mean maximum indoor temperature of the last fortnight (Colony size = exp. ($8.21532 - 0.465686*$ Mean maximum temperature last fortnight); Correlation coefficient: -0.57; R^2: 32.18%; ANOVA P-value = 0.02; F = 6.64; df = 15).

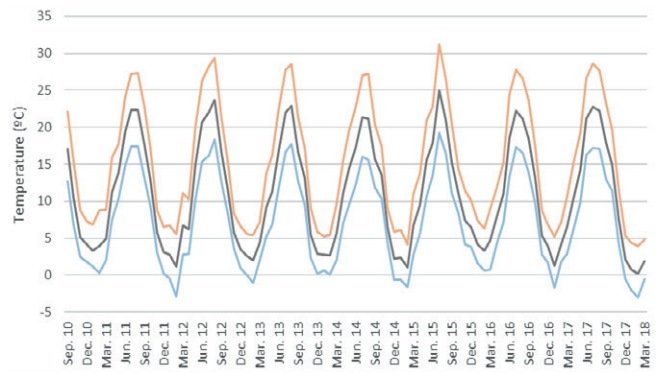**Table 3 -** Number of individuals with presence of whitish spot between the ears in relation to the total observed**.**

	Total	Presence	% Presence
13/12/2008	2	2	100.00
14/03/2009	71	59	83.10
12/10/2009	50	43	86.00
28/11/2009	162	127	78.40
28/03/2010	3	3	100.00
11/12/2010	158	116	73.42
28/02/2011	112	87	77.68
29/05/2011	2	2	100.00
11/12/2011	172	146	84.88
10/03/2012	7	7	100.00
22/09/2012	15	10	66.67
16/12/2012	407	340	83.54
24/03/2013	331	289	87.31
20/12/2013	243	212	87.24
09/03/2014	114	100	87.72
01/06/2014	7	7	100.00
06/12/2014	105	98	93.33
28/03/2015	125	114	91.20
29/08/2015	1	1	100.00
20/12/2015	219	208	94.98
02/10/2016	12	12	100.00
20/11/2016	12	8	66.67
21/12/2016	2	2	100.00
25/02/2017	1	1	100.00
06/12/2017	50	43	86.00
11/02/2018	20	17	85.00
29/03/2018	27	26	96.30

Fig. 4 - Mean temperatures and monthly ranges outside the cave during the sampling period. Grey: mean; brown: mean of maxima; blue: mean of minima.

Fig. 5 - Mean temperatures and its monthly ranges inside the cave during the sampling period. Grey: mean; brown: maximum mean; blue: minimum mean. Missing data was due to datalogger failures.
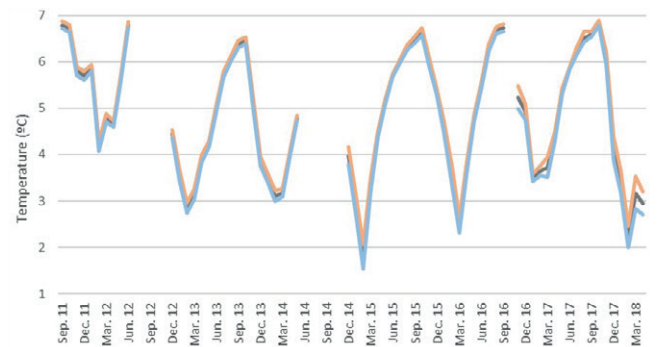
The humidity inside the cave was permanently high, saturated or close to saturation (98.54% mean ± 3·16 SD), so WVPD was zero or very close to it. Across the whole survey period, the WVPD was 1.02 hPa ± 2.23 (mean ± SD), which represents 98.56% of the SWVP. Its mean daily oscillation was 0.70 hPa ± 2.40 (mean ± SD). These values had no statistically significant effect on colony size (P-values ranging from 0.39 and 0.58). As with the temperature data and coincident with them, there was data loss due to datalogger failures.

Bats and indoor climate: Effect on clustering

Bats were distributed in the cave individually or, more commonly, in compact clusters (Table 5) with predominantly rounded or oval shapes of different sizes, which were located on the ceilings and on the sloping walls of the main room, rarely occupying cracks or cavities. The largest cluster consisted of 664 bats (16/03/2014). The size of clusters for all samples, excluding solitary individuals, was 35.50 bats ±

76.54 (mean ± SD). On the other hand, the mean of solitary individuals per census was 6.56 bats ± 4.76 (mean ± SD), ranging from 0 to 19 individuals (Fig. 6).

Cluster size was inversely related to mean temperature and directly related to mean WVPD for all periods evaluated between 1 and 15 days (Fig. 7A). Specifically, the best fit determined by AIC was found for the mean temperature and mean WVPD of the previous 15 days, with WVPD showing a somewhat greater effect than temperature (Fig. 7B & 7C), and no interaction between the two variables ($\chi^2 = 1.3$, P = 0.258). In the case of the minimum values of both factors, their effect was significant only in the periods of 1, 2 and 3 days, while for the maximum values it was significant for 3, 5, 10 and 15 days (Fig. 7A).

Potential alternative hibernation shelters

In Sierra de las Nieves National Park, in a chasm 350 m from our cave, a deposit of 35 cranio-mandibular bones of undetermined age was found, most of them belonging to *M. blythii* (CBL: n = 9; range: 20.0 - 20.9 mm; CM^3: n = 11; range: 8.3 - 9.3 mm; mandible: n = 18; range: 15.6 - 16.9 mm) and a small proportion (2.0%) of *M. myotis* (CM^3: n = 1; 10.0 mm), but no bats were currently present. On the other hand, in another chasm located in the Sierra Tejeda Natural Park, in addition to a deposit of osteological material of undetermined age of the species *M. blythii* (CBL: n = 43; 19.4 - 20.9 mm; CM^3: n = 63; 7.9 - 9.2 mm; mandible: n = 9; 16.1 - 16.9 mm), 11 hibernating *M. blythii / M. myotis* were found.

Table 4 - Number of individuals observed in each census and monthly mean. *The data corresponding to March 2014 – 15, when two censuses were conducted, is averaged to the mean.

	2007-08	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	2017-18	Mean
Sep.					58	105						81.5
Oct.			286							22		154.0
Nov.			632							216		424.0
Dec.		782		692	1032	907	814	920	804	217	320	720.9
Jan.										377		377.0
Feb.			995	1253						663	454	841.2
Mar.		883	1517		876	1121	1174*	1024			370	995.0
Apr.	295									459		377.0
May.			116	46								81.0
Jun.		0	3		5		44		0	0		8.7
Jul.		0										0.0
Aug.	4							2				3.0

Table 5 - Percentage of the colony partitioned according to cluster size, grouped in five different sizes ranges depending on the number of individuals, mean cluster size (± SD), and colony size for each date (n).

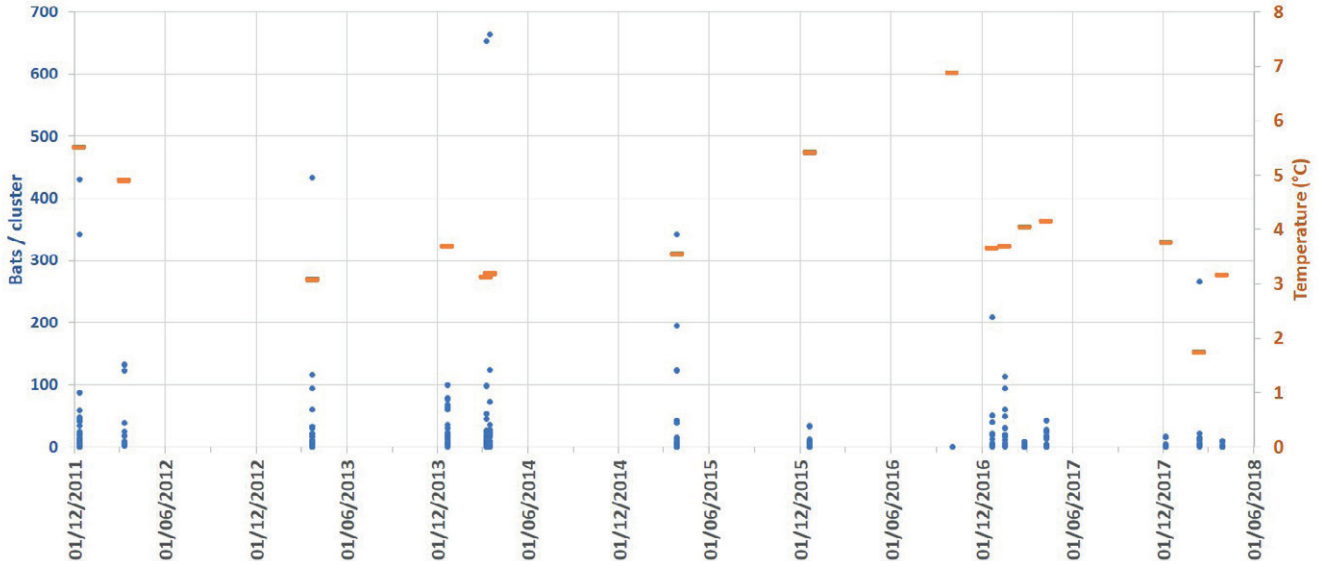|	Number of individuals in the cluster						
	1	2-5	6-50	51-100	>100	Mean (± SD)	n
11/12/2011	1.4	3.5	60.8	34.3	0.0	21.55 (± 25.81)	1032
10/03/2012	0.1	1.5	10.2	0.0	88.2	79.54 (± 153.58)	876
24/03/2013	0.5	3.8	23.8	0.0	71.8	33.79 (± 79.56)	1121
20/12/2013	0.0	0.9	32.1	67.1	0.0	32.56 (± 30.77)	814
09/03/2014	0.6	2.6	24.6	17.6	54.6	31.47 (± 105.52)	1196
16/03/2014	0.6	1.7	22.8	6.3	68.5	38.40 (± 121.03)	1152
28/03/2015	1.9	4.0	17.6	0.0	76.6	37.22 (± 76.67)	1024
20/12/2015	0.6	1.4	32.3	25.6	40.0	33.58 (± 47.49)	804
02/10/2016	68.2	31.8	0.0	0.0	0.0	3.50 (± 2.12)	22
21/12/2016	4.1	6.0	89.9	0.0	0.0	16.00 (± 11.61)	217
15/01/2017	1.1	4.0	24.4	0.0	70.6	28.69 (± 71.57)	377
24/02/2017	1.1	4.1	15.8	10.6	68.5	32.80 (± 71.61)	663
09/04/2017	1.5	1.5	50.5	46.4	0.0	26.59 (± 36.13)	459
06/12/2017	0.9	0.0	5.3	42.8	50.9	45.29 (± 55.59)	320
11/02/2018	1.3	2.4	18.7	11.9	65.6	40.73 (± 56.48)	454
29/03/2018	1.1	2.4	48.6	47.8	0.0	33.27 (± 29.89)	370

Fig. 6 - Number of individuals per cluster (excluding solitary) (blue dot) and indoor temperature (°C) (brown line) in each census.
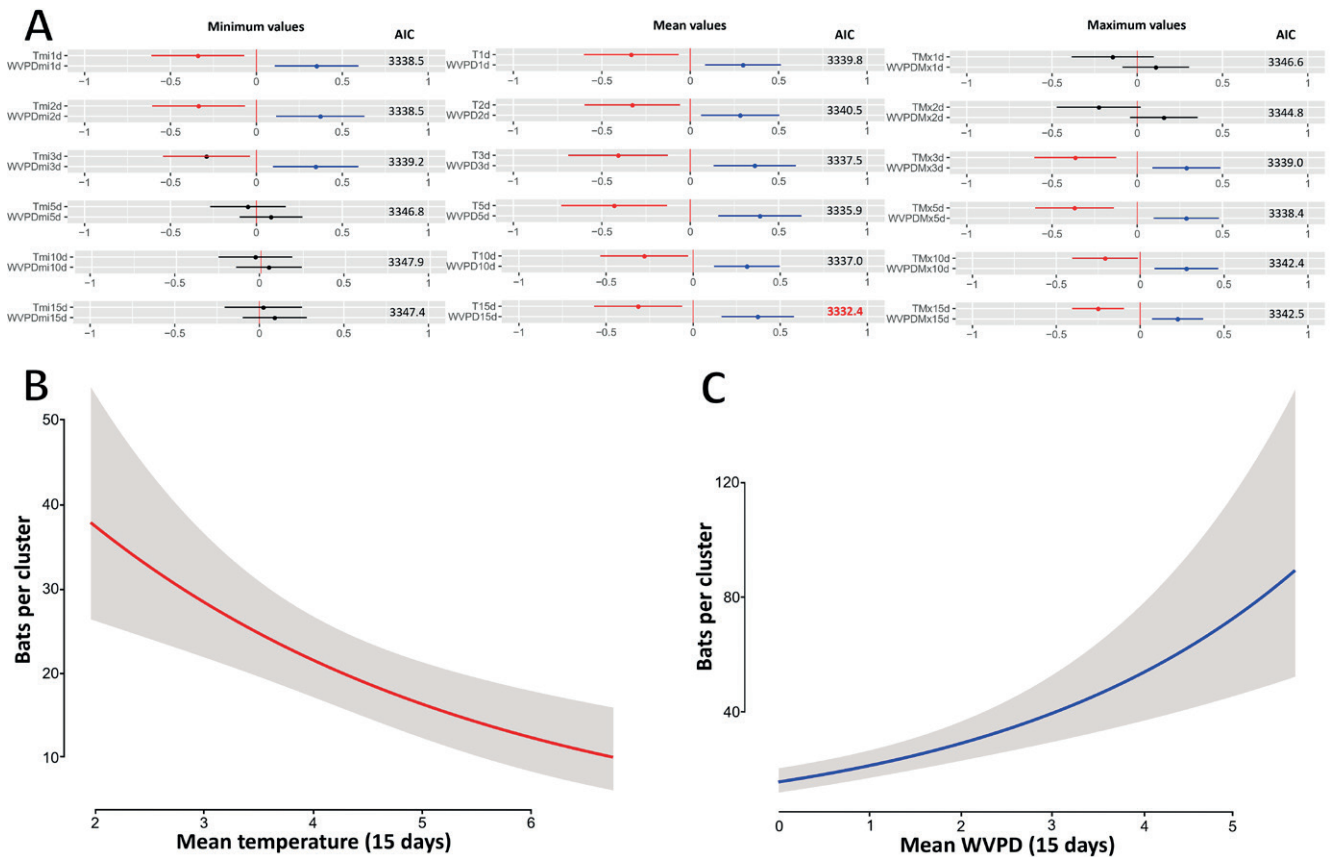

Fig. 7 - A) Forest plot of standardized estimates (± SE) for each of the 15 GLMMs run for minimum, mean, and maximum temperatures and WVPD values calculated as the mean for 1, 2, 3, 5, 10 and 15 days before sampling. AIC is shown for each model marking the lowest value (selected model) in red. Blue and red indicate statistically significant positive and negative estimates, respectively (GLMM, $P < 0.050$), and black indicates non-significant estimates (GLMM, $P > 0.050$). Predicted effect (±95% CI) of **B)** mean temperature ($\chi2 = 6.0$, d.f. $= 1$, $P = 0.014$) and **C)** mean WVPD ($\chi2 = 12.3$, d.f. $= 1$, $P < 0.001$) on bat cluster size within the selected model (mean for the 15-day period preceding sampling).

DISCUSSION

Morphological analyses of the bats in the colony have confirmed that it is composed mainly of *M. blythii*, and it is currently the only known deep hibernating colony of this species in the Iberian Peninsula. It should be pointed out that, in the Peninsula, only in Portugal some winter aggregations follow a similar pattern (Palmeirim 1999) and the most important one, located in the south of the country, supported approximately three hundred roosting bats in 2010 (ICNF 2014). This cave, 40 m deep and morphologically similar to the one in our study, is dug in Jurassic dolomites at an altitude of 467 m a.s.l. (Viegas 2006, Regala et al. 2018). Temperatures of approximately Its 15 °C (Lindberg 1962 in Regala et al. 2018) most likely prevent a state of deep hibernation from being reached, maintaining a state of daily torpor (Ransome 1990, Neuweiler 2000, Speakman & Thomas 2003). In other areas of the Southern peninsular, the scarcity of sufficiently cool caves is considered a limiting factor (Palmeirim & Rodrigues 1991). The data available, so far, on *M. blythii* hibernation in Spain are scarce and indicate that the species spends the winter scattered or in small groups in caves and mines (Romero 1990, Ibáñez 1998, Alcalde & Escala 2000), as in Sierra Nevada, Southern Spain, where some individuals have been found hibernating at 2100 m a.s.l. (Nogueras & Garrido 2007). In our cave, the mean temperature during the hibernation period (November to April) was 4.01 °C, in accordance with the temperature ranges found at higher latitudes (Gaisler 1970, Schober & Grimmberger 1989). Similar temperatures were found in the only two alternative shelters with the presence of live individuals or skeletal remains in the area. These caves, which are uncommon, act as cold-air traps that maintain mean annual temperatures lower than those of their surroundings (outside annual mean: 11.01 °C) (Balch 1900, Luetscher & Jeannin 2004).

Hibernation of *M. blythii* in other European or Middle Eastern populations ranges from October to April (Topál 1956, Roué 2004, Benda et al. 2012). In our case, it ranges from November to April, if we consider only the period of greatest cave use, or from September to May, if we consider the whole period of occupancy. Regarding the factors that influence the duration of hibernation, the literature mentions factors such as climate, sex or age (e.g., Daan 1973, Twente et al. 1985, Thomas & Geiser 1997, Berková & Zukal 2010, Czenze et al. 2017). We observed that the process of occupation of the roost by the colony is gradual, while its abandonment is faster, but we do not know the causes that produce this asymmetry.

From the 2016-17 hibernation season onwards, a decreasing trend in the number of individuals in the colony has been observed (Table 4). As the conditions in the roost have remained stable, it can most likely be attributed to external factors related to the general decline for both *M. blythii* and *M. myotis*, especially the first one (Garrido-García & Nogueras 2007, Nogueras & Garrido 2007, Consejería de Medio Ambiente 2009, 2010, 2011; ICNF 2014, Consejería de Medio Ambiente y Ordenación del Territorio 2018). Following the period covered in the present study, we have observed a recovery in subsequent winter seasons, when the following populations have been reached: 2019 - 2020: 721; 2020 - 2021: 762; 2021 - 2022: 814 individuals (pers. obs.).

Bats were mostly distributed in groups of different sizes, ranging from pairs to several hundred. Our results indicate that mean temperature and mean WVPD influenced cluster size. In the case of temperature, an inverse relationship was found, consistent with the arousal hypothesis, according to which clustering minimizes energy losses during arousals and subsequent euthermic periods (Boyles et al. 2008). In addition, given that the temperatures in the cave are relatively stable (range: 0.21 °C ± 0.21 SD) with little fluctuation, we reject the alternative hypothesis of thermal inertia (Boyles et al. 2008). On the other hand, in the case of the minimum values of both factors, clustering is only significantly affected in the closest periods (1 - 3 days), while for the maximum values the effect occurs in more distant periods (3 - 15 days). In any case, since the climatic data were taken at a single point within the cave, it is not possible to determine how microclimatic differences could have influenced the distribution of the bats (Boyles et al. 2017).

Water conservation is a key factor for determining cluster formation during hibernation (Davis 1970, Thomas & Geiser 1997). Boratyński et al. (2012), in *M. myotis*, consider ambient WVP more relevant than temperature, establishing an inverse relationship between WVP and cluster size. Our results are in accordance, both in terms of the influence of this factor, and its greater relevance in relation to temperature.

Solitary bats accounted for only 1.0% of the total population and their spatial distribution in the cave did not appear to differ from grouped individuals. Within their scarce representation, their highest proportion was reached outside the hibernation period when the population is very small, between the months of May and October (Fig. 4). This coincides with the results obtained by Boratyński et al. (2012) who attributed it to the tendency to form clusters when body reserves decline (Kokurewicz 2004).

In general, temperate bats, are subjected to a more intense selection process the higher the latitude due to increasingly cold conditions through winter, a situation that cave-dwelling bats resolve by hibernation (Strelkov 1969). On the other hand, in the south they are subjected to selective pressure due to the difficulty in finding shelters that allow deep and prolonged hibernation (Kunz 1982). This process may be aggravated by the increase in temperature due to climate change. The Intergovernmental Panel on Climate Change (IPCC) forecasts an increase in global mean surface temperature for the period 2041 - 2060 of between 1.6 ± 0.4 °C in the minimum emissions scenario and 2.4 ± 0.5 °C in the maximum emissions scenario (IPCC 2021). In the Mediterranean region, increases are likely to be even higher than global averages (Seager et al. 2014, IPCC 2021). The average temperature inside the caves, as a rule, coincides with the local annual average temperature of the outside atmosphere (Badino 2010). Therefore, global climatic changes may also influence the subterranean temperature and, consequently, the associated WVPD (Anderson 1936, Baudinette 1972, Webb et al. 1995). This influence, due

to the thermal inertia of the mountain and depending on the particular characteristics of each cave, can be delayed by years (Badino 2004, Luetscher et al. 2008, Domínguez-Villar et al. 2015). In our case, given the morphological characteristics of the cave, its behavior as a cold-air trap and its isolation, it seems unlikely that the global increase in temperature will have an immediate effect inside. To know what the climatic behavior of the cave will be in the face of global warming, a long-term monitoring effort would be necessary. As a working hypothesis, it can be expected that a delayed increase in temperature and associated WVPD will likely lead to a phenological delay with a warmer exterior (Meyer et al 2016). If, on the other hand, the increase is immediate, it would produce a shortening of hibernation (Stawski & Geiser 2012) that would remain in synchrony with the outdoor. Both assumptions represent a threat to an exceptional and fragile roost that hosts the only known case so far of a deep hibernation colony of *M. blythii* in such Southern latitudes, whose population has a narrow or null margin of choice for alternative roosts with similar characteristics.

ACKNOWLEDGEMENTS

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