Galemys, 27: 1-12, 2015
ISSN 1137-8700
e-ISSN 2254-8408
DOI: 10.7325/Galemys.2015.A1

Roosting behaviour and phenology of the Lesser horseshoe bat (*Rhinolophus hipposideros*) in a breeding colony in Sintra, Portugal

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Abstract

Almost all bats are exclusively nocturnal. However, the activity patterns throughout the night can differ between species and also within the same species from place to place. *Rhinolophus hipposideros* (Bechstein, 1800) has suffered a severe decline in many countries along its distribution area, including Portugal. In this study we aimed to describe the roosting behaviour during spring, summer and autumn of one of the largest maternity colonies of *R. hipposideros* known in Portugal. We analysed roost occupancy, activity patterns inside the roost, birth periods, and post-natal behaviour. We also tested the influence of ambient factors on the patterns of roost use. All information was obtained through the analyses of video recorded using cameras placed inside the roost, while climatic data were gathered using a meteorological station. We found that this roost has higher occupation during August and that in October it is only occupied during the night. Females gave birth from late May to early June, and in August juveniles became independent and undistinguishable from the adults. This colony presented a bimodal pattern of activity throughout the months of the study, which seems to be related with sunset and sunrise. Our results indicate that individuals of this colony prefer higher temperatures, but low humidity and wind speed to leave the roost.
Key Words: *Rhinolophus hipposideros*, paternal care, roost use, Portugal.

Resumen

Casi todos los murciélagos tienen hábitos nocturnos pero los patrones de actividad durante la noche pueden variar entre las especies y dentro de la misma especie en diferentes lugares. El murciélago pequeño de herradura *Rhinolophus hipposideros* (Bechstein, 1800) sufrió un severo declive en muchos países de su área de distribución, incluyendo Portugal. En este estudio, se pretende describir el comportamiento de refugio durante la primavera, verano y otoño de una de las colonias de cría más grande de *R. hipposideros* en Portugal. Asimismo, se analizó la ocupación y los patrones de actividad en el refugio, el periodo de nacimientos, y el comportamiento postnatal. También se comprobó el efecto de los factores ambientales en el uso del refugio. Toda la información se obtuvo a partir del análisis de los videos grabados en el interior del refugio. Los datos climáticos se obtuvieron mediante una estación meteorológica. Se observó que el refugio tiene una mayor ocupación durante el mes de agosto y que en octubre sólo se ocupa durante la noche. Las hembras dan a luz entre finales de mayo y principios de junio, y en agosto los juveniles parecen ser independientes e indistinguibles de los adultos. Esta colonia presenta un patrón bimodal de actividad que parece estar relacionado con el atardecer y amanecer. Nuestros resultados también indican que esta colonia de murciélagos prefiere altas temperaturas pero baja humedad y una cierta velocidad del viento para abandonar el refugio.
Palabras clave: *Rhinolophus hipposideros*; cuidado paternal; uso de abrigo; Portugal.

Introduction

The destruction of feeding habitats and suitable roosts for bats increased exponentially during the last few decades. Partially in result of this, about of 51% of all chiropteran species are listed as either critically endangered, data-deficient, vulnerable, or near threatened (Frick *et al.* 2010, Hutson *et al.* 2001).

Roosts play important roles during the life of bats (Kunz & Lumsden, 2003; Kunz, 1982). Hence, to understand how bats use them and which are the factors that influence these patterns could contribute to the development of management plans to ensure their survival (Fenton 1997) specially during critical periods of their annual cycle as hibernating and maternity. The development of pregnancy and the growth of juveniles are heavily influenced by the quality of maternity roosts. These kinds of roosts are important social units (Boye & Dietz 2005) and the availability of suitable roosts with proper microclimate can promote different degrees of fidelity to these sites (Kunz 1982). Indeed, in many species, nursery colonies are quite faithful to their location, returning every year to the same site (Lewis 1995), a tendency called natal philopatry.

The lesser horseshoe bat, *Rhinolophus hipposideros*, is the smallest horseshoe bat in Portugal (Palmeirim 1990) and even in Europe (Arlettaz *et al.* 2000, Ibáñez 1998). The mating of this species usually occurs between September and November but can continue during hibernation, even with torpid females (Krutzsch 2000, MacDonald & Barret 1993). As many other Holartic bats, e.g. *Rhinolophus ferrumequinum* (MacDonald & Barret 1993) and *Myotis lucifugus* (Fenton & Barclay 1980), each female gives birth to a single young per year and juveniles become completely independent at six or seven weeks of age (MacDonald & Barret 1993). Besides caves, this species also uses large human infrastructures with low disturbance and lighting levels where it is found solitary or in small colonies. Comparatively to other cave dwelling species, this is the one that presents lesser association with other species (Palmeirim & Rodrigues 1992). During breeding season warmer roosts but with different microclimates are clearly preferred (Schofield 1996).

Many studies demonstrated that low temperatures may delay, reduce or avoid the foraging periods of many insectivorous bat species (Anthony *et al.* 1981, Catto *et al.* 1995, Maier 1992). In fact, in Portugal, temperature and bat activity seem to be positively related (Amorim *et al.* 2012), a pattern also described in another locations (Vaughan *et al.* 1997, Erickson & West 2002) which may suggest that temperature is a good predictor of bat activity. High levels of humidity have been reported to provide good conditions for bats to forage (Amorim *et al.* 2012) possibly because high levels of humidity increase the abundance of insects (Roche 1997). Wind and precipitation are also considered important factors affecting the foraging period of bats (Parsons *et al.* 2003, Amorim *et al.* 2012, Erickson & West 2002). For example, in Italy, strong winds decrease the activity of *Myotis daubentonii* and *M. capaccinii* (Russo & Jones 2003) and, in Ireland, *R. hipposideros* seems to be negatively affected by heavy rain (McAney & Fairley 1988).

In this study, we intend to describe roosting behaviour and phenology of a breeding colony of *R. hipposideros* in Midwest Portugal and we also try to understand how environmental factors affect roost occupancy. We hypothesise that under such suitable conditions, i.e. high temperatures and humidity and low wind speed and precipitation, bats spend more time outside the roost during the night. Specifically, the main goals of our study were: (1) to define monthly patterns of occupancy and activity of adult and juvenile *R. hipposideros* in the Sintra roost using video recordings; (2) to define the period of births and to describe pup behavioural patterns along the first weeks of life; and (3) to examine how external environmental factors influence the time spent outside the studied roost through the night.

Materials and Methods

Study Area

This study was conducted in Sintra, Midwest Portugal (Fig. 1) in a maternity colony of *R. hipposideros* that currently occupies an infrastructure of the "Quinta da Regaleira". At this time, it is considered one of the largest maternity colonies of this species in Portugal despite the fact that it has suffered anthropogenic pressures that forced the colony to change its location.

In 2001 a colony of *R. hipposideros* with about a hundred individuals was found in the Monserrate Palace, Sintra. In 2008, the palace suffered restoration works that put in risk the persistence of the colony. To minimize the negative impacts, the Sintra Speleological Group (AES) tried to create similar temperature conditions in an old infrastructure in "Quinta da Regaleira" (226 meters of altitude) located at 2 km from the Monserrate Palace, to encourage bats to move to this place. This room with 12 m², called in this work "old roost" (Fig. 2a), was selected because a small colony of about 40 individuals of the same species already existed in it. The measures taken to guarantee the continued use of the roost consisted mainly in the increase of temperature inside the roost by the use of an oil heather. To create different microclimates inside the roost, a shelf was placed close to the ceiling making a kind of small room, to further increase the temperature in this area. To monitor the colony, a remote infrared video camera system was installed inside the roost. In 2009, mainly because this structure also needed to restoration works, new efforts were initiated to change the location of the colony to a neighbouring room only 30 meters away. This new roost had 22.75 m² (Fig. 2b). Two oil heaters, one shelf and a remote infrared video camera system were placed in it but the colony only changed its location to this new room in 2011. The artificial heating of the roost was the crucial factor that enabled its use by *R. hipposideros* since this species needs warm roosts during pregnancy and unsystematic records

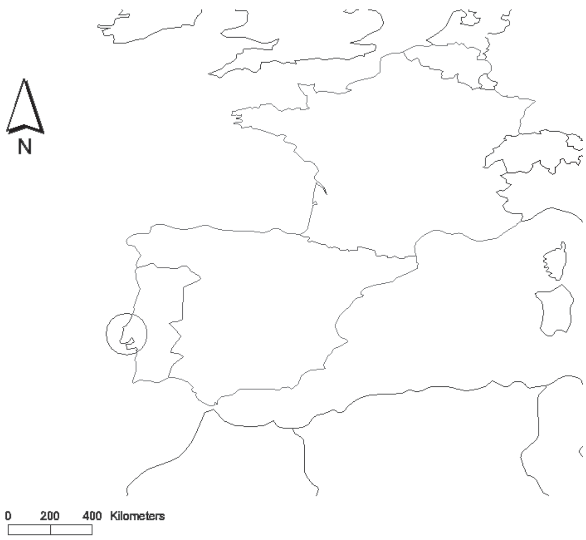

Figure 1. Approximate location of the study area (Sintra, Portugal).

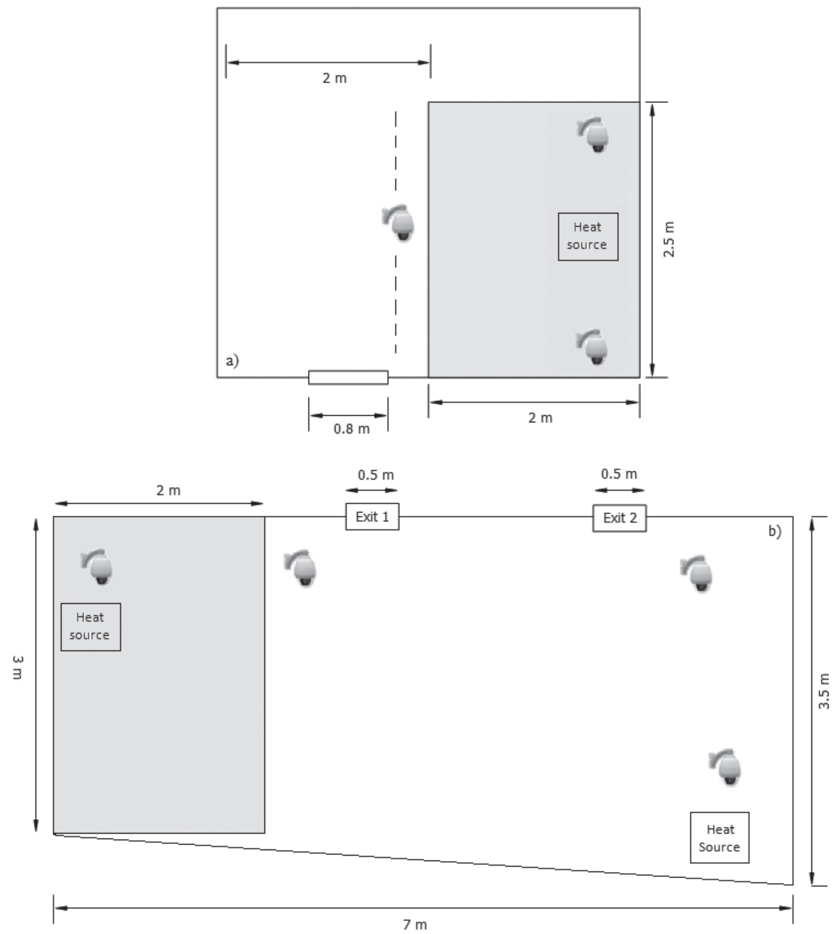

Figure 2. Schematic representation of the studied roosts: **a)** "ancient roost"; **b)** "new roost". The light grey space corresponds to shelves placed in the roosts; because oil heaters are set beneath these shelves in both the "ancient roost" and the "new roost", these are warmer areas. The cameras represent the areas that were video recorded. Both roosts are 5 meters high.

indicate that temperatures within the roost never went below 16ºC. Because no bats were marked, there is no way of knowing if the increase in the number of bats in "Quinta da Regaleira", that may presently reach up to 150 individuals at least during the summer, was due to the migration of bats from the Monserrate colony. Nonetheless, "Quinta da Regaleira" currently represents one of the major known breeding colonies of *R. hipposideros* in Portugal.

The area surrounding the "Quinta da Regaleira" roost is characterized by typical Mediterranean woodland, dominated by species such as *Quercus* spp., *Pinus pinea* and *Ilex aquifolium*, though plantations of *Pinus pinaster* and *Cupressus lusitanica*, as well as many exotics species as *Eucalyptus globulus*, *Acacia* sp., *Pittosporum undullatum*, *Hakea salicifolia*, *Hakea sericea* and *Aillanthus altissima* are also present. The dense vegetation cover present is probably a response to the specific microclimate of the Sintra mountain range, where high relative humidity levels, cold temperatures for the region (average: 14.8ºC/year) and significant levels of rainfall for a Mediterranean region (average: 1006 mm/year; (Lousã *et al.* 2005) are common all year round, though rainfall is more frequent during the winter.

Analysis of the video recordings

We analysed video recordings of the roost from March to October of 2010 and 2012. For statistical analyses we only considered 29 days of July (n = 7 days), August (n = 11 days) and October (n = 11 days) in 2010 and 69 days of March (n = 4 days), April (n = 29 days), May (n = 21 days), June (n = 1 days), July (n = 3 days), August (n = 6 days) and October (n = 5 days) in 2012 because these were the days with recordings from all cameras during 24 hours a day.

To describe roost occupancy we counted the number of adults and juveniles in the roost. To avoid the risk of counting the same individual twice, the video recordings of different cameras were analysed simultaneously, through the GeoVision Remote ViewLog program. Thus, we could see if new individuals arrived or departed the roost or if only changed their location inside the roost. We obtained nightly patterns of roost use by calculating hourly average and to describe the monthly pattern we used the previous data to calculate monthly average for the night during 20h and 08h. The mean number of adults and juveniles during the day was obtained through the mean of individuals in the roost during 09h and 19h. To describe roost activity we counted the number of flights inside the roost during day- and night-time using a similar approach to the one used by Weinbeer *et al.* (2006).

Climatic variables

Weather conditions data were obtained from the meteorological station closest of the roost, the Mira-Sintra station (http://www.wunderground.com/). We used the following climatic parameters: temperature (ºC), wind velocity (km/h), humidity (%) and rainfall (mm). To correlate these data with roost occupation we calculated hourly average for the respective days that we used in recording analyses.

Data analyses

We tried to understand how climatic variables affected the number of individuals in the roost overnight slightly before the first and after the second emergence time (between 19h and 08h) through the use of Generalized Linear Models (GLM). The ratio between the number of individuals in the roost overnight and the total number of bats in it during the day was used as dependent variable. The climatic parameters were used as independent variables. To avoid data multicollinearity, i.e. the correlation between independent variables, we performed the hierarchical partitioning analysis using the 'hier.part' package in R (Walsh & Nally 2013). Additionally, we performed a randomization test (100 and 1000 randomization) using the 'rand.hp' function to test which variables should be included in the model. The climatic variables tested were temperature, precipitation, humidity, pressure and wind speed. These models were developed using data from the months with higher occupancy levels, specifically July and August 2010 and June, July and August 2012. Analyses were done in R version 3.1.3 (R Core Team 2015).

Results

The studied roost was occupied mainly as a summer roost during both day and night-time. At the beginning of March, females started to settle in the roost and gradually leave from September to October, when the roost was mostly occupied as a night roost.

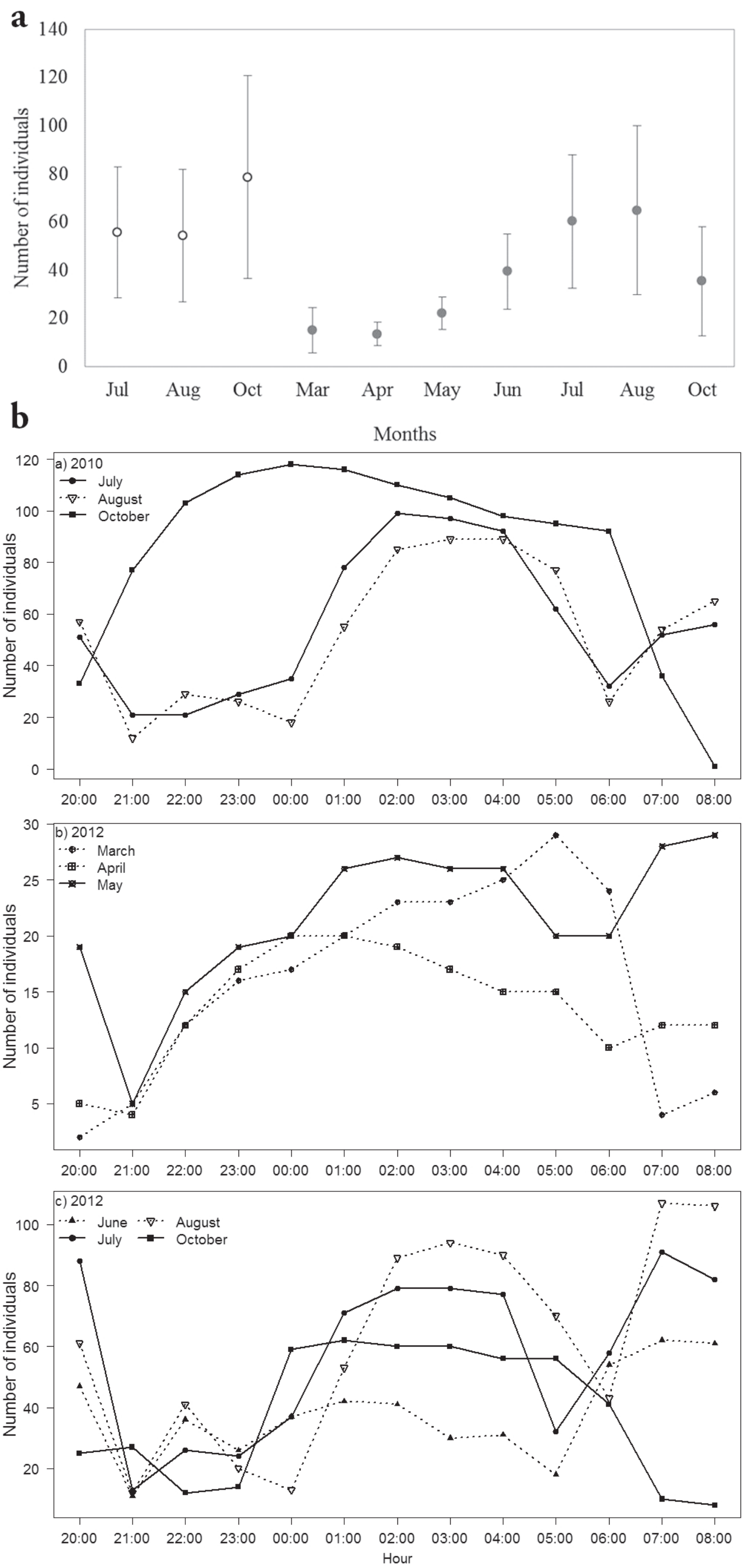

Figure 3. a) Average number of individuals that occupied the roost during 20h00 and 08h00, in the studied months: July (n=7 days), August (n=11) and October (n=11) of 2010 represented by white circles and March (n=4), April (n=29), May (n=21), June (n=1), July (n=3), August (n=6) and October (n=5) of 2012 represented by grey circles. **b)** Hourly average of the number of individuals that occupied the roost during night-time in the studied years: a) July (n=7 days), August (n=11) and October (n=11) of 2010; b) March (n=4), April (n=29) and May (n=21) of 2012 and c) June (n=1), July (n=3), August (n=6) and October (n=5) of 2012.

Because some of the equipment experienced technical problems and image capture was not possible for some days, we chose to analyse only the days with records of the whole 24 hours in all the main cameras of the roost. From March to September 2010 we gathered recordings from only two cameras, and also from a third camera in October. In 2012 we gathered images from three cameras in the main room of the roost and from another one in the smaller room created by the shelf placed close to the ceiling.

Species composition

The colony is almost exclusively composed by *R. hipposideros*, but there were some sporadic visits of *Plecotus* sp. during 5 days in October 2010 and 1 day in August 2010. The duration of these visits changed from some minutes to a maximum of 4 hours. Sometimes, when this happened, some distress of the individuals of *R. hipposideros* was observed, increasing the number of flight activity inside the roost and often avoiding the proximity with *Plecotus* sp.

Night time occupancy of the roost

Figure 3a shows that the number of individuals occupying the roost during night-time was higher in summer months and in October 2010. Figure 3b shows the variation in the number of individuals that occupied the roost during night-time per month in the two studied years, 2010 and 2012. In July and August of 2010 the number of individuals using the roost during the night seems to be very similar with a maximum of 99 individuals (st.dev= 39, n=7) at 02h in July.

During spring months of March, April and May 2012 the number of bats in the roost was lower than in summer months, reaching to only 29 individuals (st.dev = 6, n=4) in March 2012. After June the number of individuals in the roost increased gradually reaching its peak in August.

In October, the only truly representative month of the autumn season, more individuals were recorded in 2010 than in 2012. During this period the roost was occupied only during the night and the monthly mean number of individuals inside the roost reached 118 individuals (st.dev. = 21, n= 11).

During July and early August it was possible to recognize juvenile bats (Fig. 4). More juveniles were counted during July of both years than in August possibly because it became very difficult to distinguish juveniles from adults using video recordings. During night-time, the number of juveniles in the roost seemed to follow a very similar pattern to that showed by adults.

Daytime occupancy of the roost

The daytime was considered the period between 09h and 18h. During this period the number of individuals occupying the roost was constant throughout and increased along the studied months except in October of both years (Fig. 5).

In July and August 2010, the maximum number of individuals in the roost was 72 and 80, respectively. In March, April and May 2012 the maximum number of individuals during daytime was very low with only 7, 12 and 30 individuals in each month. The higher number of individuals during the considered period was registered in the summer months of 2012 with 63, 88 and 112 individuals in June, July and August, respectively. In October, the number of individuals in the roost never exceeded 2 in 2010 and 9 in 2012.

In July 2010 and 2012 it was possible to recognize a higher number of juveniles in the roost than in August of the same years (Fig. 6).

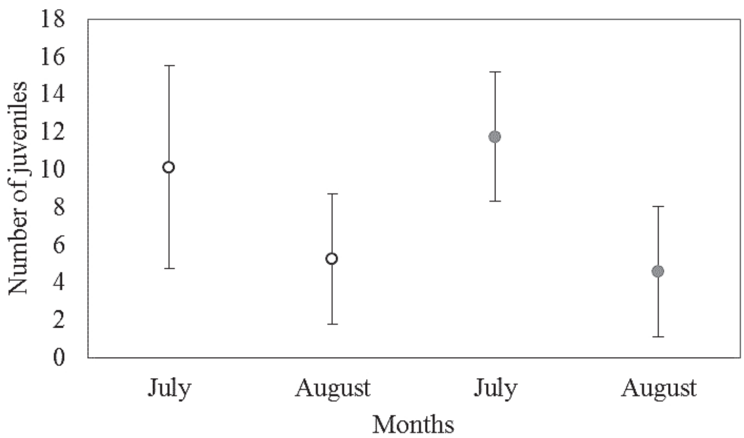

Figure 4. Average number of juveniles that occupied the roost during night-time in 2010 (white circles) and 2012 (grey circles).

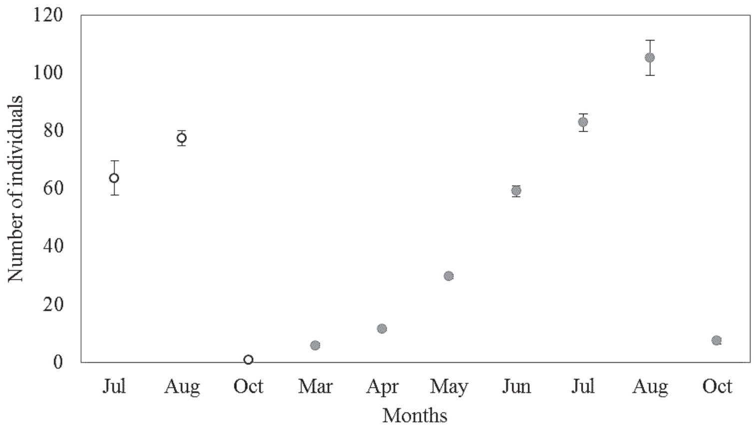

Figure 5. Average number of individuals that occupied the roost during day time, between 09h and 19h, in the studied years 2010 (white circles) and 2012 (grey circles).

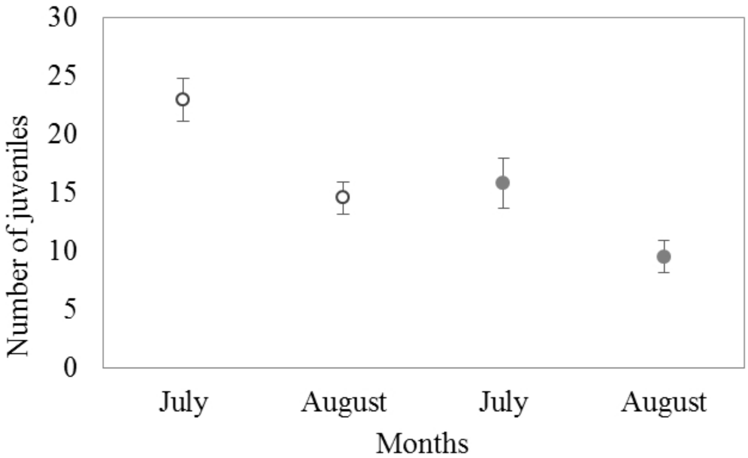

Figure 6. Average number of juveniles that occupied the roost in day-time in 2010 (white circles) and 2012 (grey circles).

Emergence time, nocturnal occupancy of the roost and flight activity

Although the first departure was more evident than the second, it seems reasonable to say that individuals of this colony had two main emergence periods (Fig. 3b). The data suggest that emergence times were related with light intensity (Fig. 7). The first departure occurred at the same time or until 1 hour after sunset. The second departure occurred between 25 minutes to 90 minutes before sunrise but in March 2010 and October 2012 they only occurred until 15 minutes after sunrise.

Bat activity, measured as the number of flights performed inside the roost, was higher at sunset and sunrise (Fig. 8) when compared with other periods. In fact, the individuals of this colony performed several flights of entry and exit of the roost before they definitely left the roost to forage.

Phenology of the breeding colony

Though we never registered a birth in the roost with the cameras, the first pups of the year were recorded on 27th of May 2010, and on 5th of June 2012, respectively. On 28th of May of 2010 a pup was observed suspended alone in the ceiling of the roost and suspended in its mother in the next day. The first flight attempts were registered four days after the first recorded pup. When females left the roost during night-time, they carried their young outside the roost or, at least, left them somewhere outside the monitored area. In July the juveniles started to practice several short flights inside the roost and, a few days later, they began to leave the roost by themselves. From August onwards it started to be difficult to distinguish the training flights of juveniles from the adult flights most likely because many juveniles would already be able to fly outside the roost. Thus, it was possible to count more training flights during July months of the studied years.

Climatic variables affecting roost occupancy during the night

The hierarchical partitioning process showed that precipitation and pressure were highly correlated with the others climatic variables, therefore they were excluded from the next analysis. So, in the

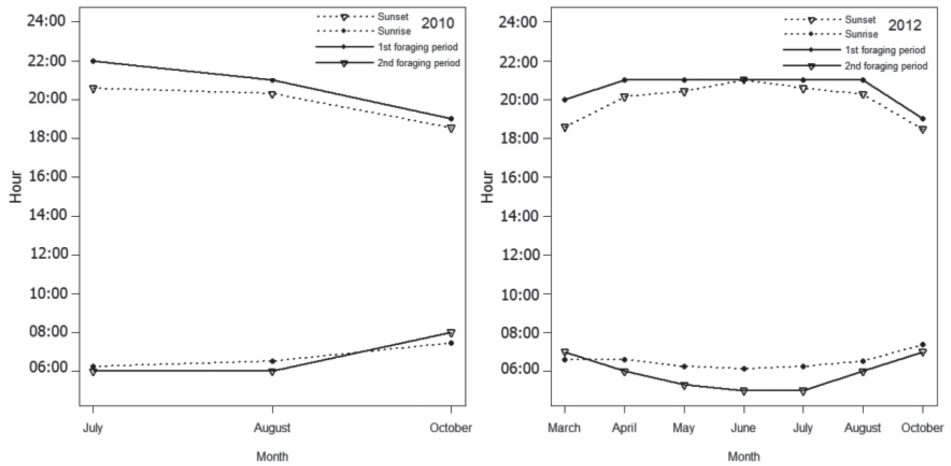

Figure 7. Foraging periods and time of sunrise and sunset during the studied months of 2010 and 2012.

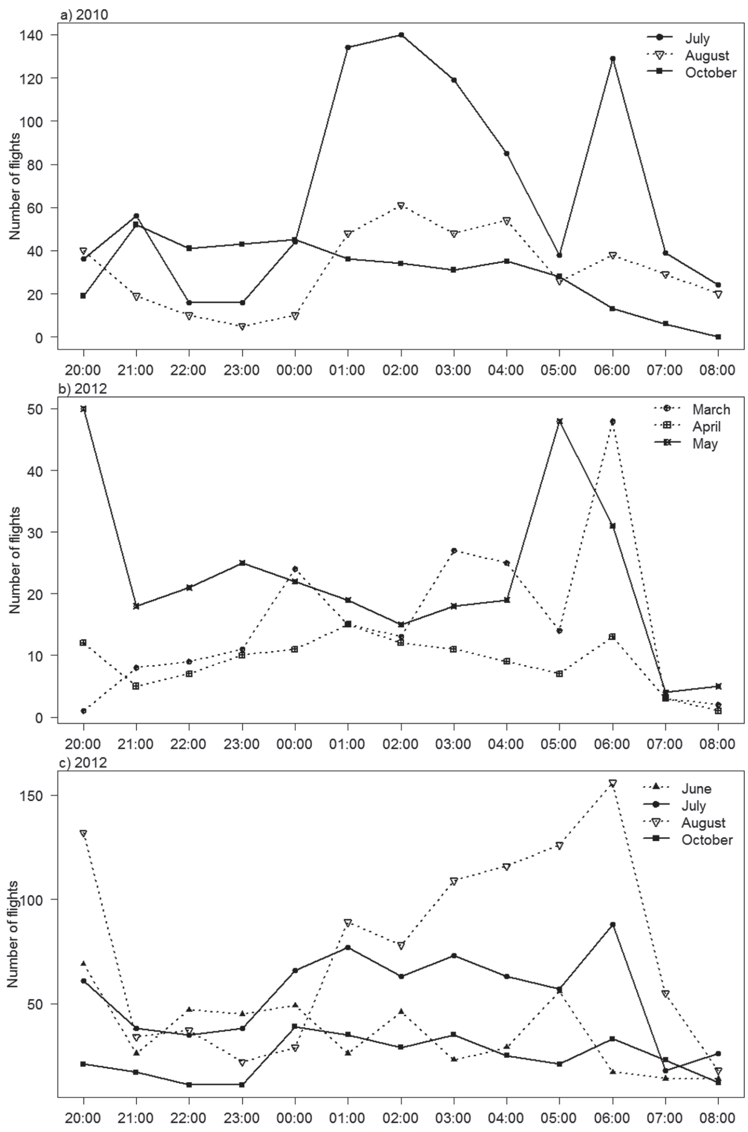

Figure 8. Number of flights in the roost during the 2 years: **a)** 2010, 'ancient roost'; **b)** 2012, 'new roost' and **c)** 2012, 'new roost'.

GLMs we used temperature, humidity and wind speed as predictor variables. All these variables seemed to explain roost occupancy overnight during the studied summer months, July and August of 2010 and June, July and August of 2012 (Table 1). Temperature was negatively correlated with the occupancy of the roost during the night while humidity and wind were positively correlated with the occupancy of the roost during the night.

Table 1. Parameters for the variables included in the GLM explaining roost occupancy overnight during the months showing the highest occupancy rates.

Variables	Estimate	Std.Error	z value	Pr(>|z|)
Intercept	4.035435	0.200082	20.169	< 2e-16
Temperature	-0.059285	0.006054	-9.792	< 2e-16
Humidity	0.007015	0.001287	5.451	5.02E-08
Wind	0.023488	0.001141	20.593	< 2e-16

Discussion

Despite some technical problems with the cameras, which reduced the number of available recordings, our data allowed us to assess how factors influence roost occupancy and patterns of activity of *R. hipposideros* in the studied nursery roost. In addition, using video recordings, no disturbance was made during this very sensitive time of the life-cycle of bats, guaranteeing that the most natural possible behaviours were registered.

Emergence time, nocturnal occupancy of the roost and flight activity

The individuals of this summer colony of *R. hipposideros* presented a bimodal activity pattern that varied seasonally. In fact, the availability of aerial insects, daylight-length (McAney & Fairley 1988, Catto *et al.* 1995), temperature (Lacki 1984, Russo & Jones 2003, Russ *et al.* 2003), and other climatic factors as rainfall (Shiel & Fairley 1999), relative humidity (Lacki 1984) and wind speed (Russ *et al.* 2003, Russo & Jones 2003) seemed to play an important role in the activity patterns of insectivorous bats. Some studies show that insectivorous bats present this bimodal pattern due to the exploitation of peaks of activity of aerial insects: a first peak in the number of insects usually occurs after dusk and a second just before dawn (Swift 1980, Rautenbach *et al.* 1988, Jones & Rydell 1994). As insect activity seems to decline in the middle of night, apparently caused by a drop in ambient temperature (Rydell 1992), insectivorous bats do not have any advantage in a continued foraging activity all night long (Rautenbach *et al.* 1988, Jones & Rydell 1994), because this would mean a waste of energy (Kunz 1973, Swift 1980, Jong & Ahlén 1991). According to Schoener (1971), under the light of the optimal foraging theory, the optimal diet is one which provides one way to obtain the greatest net energy per unit feeding. Because the availability of insects after dusk and before dawn is high, during this period bats are not required to spend too much energy to search and capture prey. Therefore they take advantage of this abundance of prey to yield maximal net energy gains and to minimize the energetic expenditures when searching for food.

Flight activity within the roost varied along the day and seemed to have a strict relation with emergence periods. Increased activity before the first departure was probably related to the fact that adult bats need to check levels of light intensity before leaving the roost (McAney & Fairley 1988, Seckerdieck *et al.* 2005, Griffiths 2007), and thus perform several flights exiting and entering the roost before they definitely departure to forage (McAney & Fairley 1988). Light sampling plays an important role in the regulation of the nocturnal activity of several bat species and it is also one way of bats avoiding predators that need of at least some light to capture their prey (Isaac & Marimuthu 1993, Griffiths 2007).

Phenology of the breeding colony

During the two years of the study, 2010 and 2012, births happened relatively early when compared with the patterns described in other locations (Schofield 1996, Reiter 2004, Ifrim 2007). In our study, births started in 27th May in 2010 while in Wales and in Romania births only started in the end of June and in the second half of July, respectively (Schofield 1996, Ifrim 2007). This is surely due to the fact that timing of parturition is strongly influenced by environmental factors, including climate and food availability. In lower latitudes, ambient temperature, and consequently prey availability, start to increase sooner in the year that in northern areas, explaining this asynchrony in birth timings between our study and those of Schofield (1996) and Ifrim (2007). Food availability is indeed a chief factor regulating the timing of parturition (Arlettaz *et al.* 2001) and late parturition may negatively affect growth and survival of young bats (Ransome & McOwat 1994). *R. hipposideros* in Sintra have thus more time to store fat reserves for hibernation, which may increase the probability of survival and breeding during their first year when compared with pups that are born later in the summer or from higher latitudes.

During the lactation period, few pups were registered in the roost during the emergence periods of the adult females, suggesting that they were carried by them. However, during some nights, a small number of females left their pups in the roost, but they did not seem to show any preference for the place where to leave the young bats, a behaviour already described by Schofield (1996) in this species and by Sano (2000) in *Rhinolophus ferrumequinum* in Japan.

Environmental factors affecting roost occupancy overnight

Manmade structures usually exhibit a marked variation in climatic parameters, especially temperature and relative humidity, in contrast to the constancy presented by underground roosts (Kunz 1973). However, though this roost is located in a manmade infrastructure, the internal temperature is somewhat controlled by the presence of a heater. Due to this, there are no significant variations in roost temperature during the breeding period, so this variable is probably not a factor strongly influencing bat activity in the studied colony.

Life strategies of bats are regulated by many factors e.g. prey availability and moonlight but climate is surely among the most important as it can affect bats directly, inhibiting some activities such as flight, foraging and mating. Indeed, it is known that bats have minimum ambient temperatures to forage, and during cold nights it has been shown that the length of the foraging periods decreases, so bats spend more time inside their roosts (Roche 1997) as occurs with the studied colony in Sintra. Ambient temperature also can influence bat activity indirectly by affecting the abundance and activity of arthropods (Taylor 1963, Anthony *et al.* 1981, Rydell 1989), which are the main components of the diet of insectivorous bats.

Flying with higher levels of humidity seems to be a behavioural strategy to reduce excess water loss and, as insects have a higher level of activity under these conditions, bats need to spend less time to search and feed on them (Roche 1997). However, we found that the studied colony increased the time spent outside the roost with lower humidity levels, probably increasing the time spent in foraging. A possible explanation for a decreasing activity with moister nights is the fact that, in our study area, nights are frequently very humid and the relation between bat activity and this variable may be unimodal rather than linear, i.e., activity is positively related to humidity only up to some point, after which it becomes too humid and bat activity decreases. However, our data did not allow us to test this hypothesis.

R. hipposideros seems to decrease the time spent outside the roost with increasing wind speed. In this case our results seem to be consistent with other studies that argue that strong winds tend to challenge bat flight. In Italy, Russo & Jones (2003) showed that the activity of *Myotis daubentonii* and *Myotis capaccinii* were clearly reduced by strong winds. Other studies, however, argue that this negative correlation is caused by the effect of wind on insect availability, because strong winds preclude the flight of insects (Peng *et al.* 1992) making them unavailable to bats that forage on flying arthropods.

Conclusions

The methodology applied in this study reveals itself a good way to study bat colonies in its natural conditions. The use of video cameras to register the behaviours during maternity period and patterns of roost use by bats allows the recording of data without any kind of human disturbance. The peak of occupation of the roost occurred during summer months which indicates that females selected this roost essentially to give birth and raise their young. During the two years of study, parturition time was between late May and early June. The emergence time of *R. hipposideros* seems to be closely related to light intensity, and is maximum near sunset and sunrise. However, activity of *R. hipposideros* also seems to be determined by other factors such as temperature and wind speed.

The conditions created inside the roost were crucial factors that enabled its use by the colony. For this reason, the efforts made until now should be maintained to protect one of the major colonies of *R. hipposideros* in Portugal.

Acknowledgments

MJRP was funded by Fundação para a Ciência e Tecnologia postdoctoral grant SFRH/BPD/72845/2010.

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Associate Editor was L. Javier Palomo