UNIVERSITÀ DEGLI STUDI DI FIRENZE

DIPARTIMENTO DI BIOLOGIA EVOLUZIONISTICA "L. Pardi"

DOTTORATO DI RICERCA IN ETOLOGIA, ECOLOGIA ANIMALE E ANTROPOLOGIA (XXV CICLO, BIO 05, BIO 07)

Behavioral ecology of bats in urban and suburban areas: an eco-ethological approach to conservation

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2012

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ABSTRACT

Human tolerant species of bats are an important constituent of the biodiversity, albeit-low, in urban and suburban ecosystems. They usually survive by taking refuge inside human artefacts and sometimes using urban green spaces as foraging areas, where they assist in regulating insect density thus providing an important ecosystem service to citizens. Their conservation is therefore particularly important also because of their status which is threatened throughout Europe.

I studied the ecology of the shelters choice of both the group of species which roost in buildings and in other human artefacts in Italy: crevice dwelling bats and those that need larger volumes like attics or cellars.

As a model species to study the latter group I chose a nursery colony of *Rhinolophus ferrumequinum* which during the spring-autumn period live in the Natural Park of Migliarino San Rossore Massaciuccoli. I monitored four different roosts that the colony can select throughout the year and I found that the internal microclimatic conditions (temperature and relative humidity) as well as the risk of predation influence significantly the choice by bats which can easily shift between their roosts in cases of necessity.

To study the preferences of crevice dwelling bats I used the bat boxes, which are artificial roosts for bats. These shelters were placed by a large amount of citizen volunteers who monitored the colonization of their roosts throughout the year. Monitoring data were collected yearly and their analysis showed a similar trend to the other group of species. Bats choose their roost to better suit their termoregulatory needs and to avoid the risk of predation, selecting the roosts placed since more time. The study, which was conducted over more years, also showed that bats learn to use bat boxes over time, colonizing them earlier in the years subsequent the first year of occupation. I also studied more in depth how the internal temperature affects the choice of roosts in crevice dwelling bats and I found that these animals likely select the roost in order to save energy during the daily torpor.

My results also showed a minor utilization of spring-autumn roosts during winter by some individuals both in crevice dwelling and in species which need large volumes. This may be related either to the urban heat-island effect or to a general climate change, at least in the strictly urban species.

I also studied the emergence and pre-emergence behavior of a nursery colony of *R. ferrumequinum* throughout the year, also focusing on the reproductive period. The nightly emergence in bats is a tradeoff between the opportunity to prey and the risk of being a prey for diurnal raptorial birds. The onset of such behaviour is regulated by an endogenous circadian rhythm which is adjusted by various ecological factors. I showed that the onset of the emergence, as well as its distribution of intensity, is influenced by the temperature and by the evening light intensity which facilitate the evening arousal and decrease the predation risk, respectively. The onset of the pre-emergence light-sampling behavior is also anticipated by the presence of pups which increase the trophic necessities of lactating females. The duration of the nightly emergence is although mainly influenced by the presence of juveniles which, leaving later than adults to avoid predation, cause a longer emergence.

The distribution of about 50000 bat boxes over five years through the "BAT BOX: be a bat's friend" project also allowed to involve people directly in a conservation programme entirely focused on bats. To raise people interest and awareness I properly talked, through public meetings, interviews and brochures, about the ecosystem services that bats perform. In particular I focused on the control of insects, thus contributing to change the originally bad attitude toward bats. Through the project I succeeded in positively influencing the behavior of people, making bats a flagship species for the sustainable control of harmful insects.

1. INTRODUCTION

Bats (Chiroptera) are an order of Mammals, which is second in species richness only to the rodents (Rodentia). Their traditional classification includes the two suborders Microchiroptera and Megachiroptera which divide on a morphological basis echolocating and not-echolocating bats, respectively, with the only exception of the echolocating *Rousettus* genus, placed in the latter suborder. Recent studies supported this classification also on a molecular basis, assuming the evolution of these groups from a common ancestor already able to fly (Simmons et al. 2008). Traditionally, Italian bat species belonged entirely to the Microchiroptera suborder, and include echolocating bats which feed predominantly on insects. However, the above cited classification has long been debated by some specialists who proposed some alternative interpretations for the phylogeny of bats. Basing exclusively on genetic evidences Teeling et al. (2005) proposed a new classification which is currently recognized as the most correct, although still debated. The two new proposed suborders divide bats in Yinpterochiroptera and Yangochiroptera. The first include all Megachiroptera bats as well as the Rhinolophidae, Hipposideridae, Craseonycteridae, Megadermatidae, and Rhinopomatidae families, while the latter include all the remnant families of the former Microchiroptera suborder. Among the four families of Italian bats, Rhinolophidae now belongs to Yinpterochiroptera while Vespertilionidae, Molossidae and Miniopteridae belong to Yangochiroptera (Lanza 2012).

Despite their newly recognized phylogenetic distance, Italian bat species share the majority of their ecological features. They all have nocturnal habits, they feed mainly on insects and use echolocation to hunt and navigate in the dark at night. Unfortunately they also share a general alarming status of conservation, supported by a significant decline in their populations all over Europe (Hutson et al. 2001; Bontadina et al. 2008). Trying to stop this generalized decline, the Convention on Migratory Species (CMS) established the European Bat Agreement (EUROBATS) which also Italy joined in 2005 to protect all the European bat species through legislation, education, conservation measures and international co-operation (UNEP 1991). Most of the threats to bats are related to the increasing human population which directly or indirectly negatively affect the habitats where bats live (Hutson et al. 2001). The human intervention on the environment often destroy or modify precious foraging areas or roosts which are necessary for the presence of bats in a given area. Forests, for example, are a key habitat for bats: some bat species live exclusively in well conserved and large enough forests with a proper density of old fissured trees where to find a roost. However, the forest management, which is usually not sustainable, does not include the presence of such trees, which are not productive and are risky for the people safety (Lacki et al. 2007). In the rural landscape, agriculture increasingly tend to change from traditional to intensive, with greater use of artificial chemicals as fertilisers and pesticides. Such not-sustainable agriculture shift caused the banalization of many rural sensible agro-silvo-pastoral systems which contained some key elements for the presence of bats such as tree lines, hedgerows and canals. High concentration of harmful chemicals also afflict bats which may accumulate them through the ingestion of contaminated food or water. Upon reaching a critical concentration of chemicals inside their tissue, bats die, usually during hibernation, when they consume the fat cells in which these compound are generally stored (Clark 1981). Also underground sites used by bats as winter hibernacula or summer roosts are often threatened by caving, tourism or the risk of sealing of old mines and caves (Hutson et al. 2001). Moreover people do not like bats and generally do not care about their conservation (Lunney & Moon 2011). People are generally scared of bats, some because of vampire myths, others for the rabies threat, yet others just because their nocturnal activity.

Nevertheless, the presence of human artefacts can favour some species of bat, and these species constitute some of the most valuable components of the degraded urban and suburban ecosystems. Some generalist species adapted to live closely to humans exploiting the productivity of the urban landscape (Threlfall et al. 2011), while the majority of the human-tolerant bat species just adapted to use human infrastructures as a roost (Lausen & Barclay 2006). Bat species which use shelters in building and other human artefacts, can be approximately distinguished in two groups in Italy: crevice dwelling and bats needing large volumes which hang from ceilings. The first, such as *Pipistrellus kuhlii*, *Pipistrellus pipistrellus* and *Hypsugo savii*, use small crevices, wall cracks, spaces between external beams and walls or behind the shutters. The latter, like *Myotis emarginatus*, *Rhinolophus ferrumequinum* or *Rhinolophus hipposideros*, use cave-like rooms such as attics and cellars (Agnelli et al. 2006). Bats living in urban and suburban ecosystems share ecological similarities and necessities with the species that live in other biomes. Therefore human-tolerant species are still afflicted by the same threats which are causing the bats decline throughout Europe. Further study to better understand the ecology of such species are thus needed to tune proper conservation measures oriented to them.

Finding an adequate roost is one of the most difficult challenge for bats. In the temperate zone, during the year bats often need different roosts to switch between each other under particular circumstances. The choice of a roost depends on seasonal requirements and is largely influenced by microclimatic factors such as internal roost temperature and humidity (Fenton & Rautenbach 1986; Churcill 1991; Entwistle et al. 1997) and the surrounding environment (Wunder & Carey 1996). During winter, bats need temperature-stable shelters in which to hibernate, like underground caves, while in the period of activity, males and females meet different needs of roost related to the daytime torpor dynamic. During the reproductive season (spring-summer) the former live solitary and need colder shelters to lower their metabolism, while the latter meet in sun exposed roosts, forming nursery colonies to warm each other (Grinevitch et al. 1995). This particular behavior allow males to save energy during the day and females to speed up the development of embryos during the period of pregnancy. During the mating period (autumn) both sexes meet each other in roosts which are often actively defended by males (Dietz et al. 2009). The roost-switching is even more frequent in those bat species which roost in less stable shelters like crevices in trees (Russo et al. 2005). This huge demand of roosts with different features throughout the year probably drove the adaptation of some bat species in using human artefacts.

Some generalist crevice dwelling bat species, originally adapted to use fissures in rocks or cavities in trees, learnt to use the many small crevices in human buildings, thus following human aggregations and becoming the most human-tolerant bat species. Italian bat species which need large volumes to roost in buildings are generally less generalist, needing well conserved agro-silvo-pastoral systems to hunt their prey (Lanza 2012). The large volumes, such as attics or cellars, that these species need to roost are also increasingly rare in human buildings, especially in big cities. The presence of such species in human artefacts is therefore often limited to the rural landscape where the destruction or renovation of old buildings is causing a major threat for the conservation of those species. One of the most studied human-tolerant species which roost in large volumes is *R. ferrumequinum*. This species has been recorded in all regions of Italy, and typically favours areas below 800 m a.s.l. characterized by habitat mosaics (Agnelli et al. 2006). It uses different roosts during different seasons throughout its range, and typically these roosts are separated by less than 30 km (Dietz et al. 2009). During winter, these bats utilize roosts in natural or artificial underground sites, with an internal temperature of 7-12 °C; while summer roosts are typically in artificial sites, such as buildings, caves and mines, and rarely in tree holes. In the UK, the range of *R. ferrumequinum* has contracted over the past 100 years and the population decline is estimated over 90% (Stebbings 1988). One of the largest nursery colonies of *R. ferrumequinum* in Italy during the activity period inhabits multiple roosts in the Natural Park of Migliarino San Rossore Massaciuccoli (Agnelli & Guaita 2010), which is five kilometers far from the city of Pisa (Tuscany, Italy). In order to understand the role of microclimatic and other ecological parameters like the risk of predation and the pregnancy status of females in the choice of roosts, I studied the dynamics in the use of the shelters throughout the year during a one-year monitoring.

Microclimatic, predation and pregnancy also affect the emergence behavior performed by bats every evening during the activity period (Kunz & Anthony 1996; Shiel & Fairley 1999; Duvergé et al. 2000; Russo et al. 2007). This behavior is a compromise between the opportunity to prey and the risk of being a prey (Fenton et al. 1994; Jones & Rydell 1994; Rydell & Speakman 1995; Speakman 1995). In fact, leaving the roost earlier in the evening exposes bats to an higher risk of predation by raptorial birds which may still be active. On the other hand, emerging later, bats risk to miss the moment of higher insect activity during the day (Racey & Swift 1985; Rydell et al. 1996) although visual acuity of diurnal predators decreases rapidly (Fox et al. 1976). Choosing an adequate timing for the nightly emergence at sunset is therefore vital for bats. This timing is mainly regulated in bats by an endogenous circadian rhythm (Erkert 1982) which is adjusted using environmental information and influenced by the physiological status. For example, prior to the emergence bats which use large volumes in building often perform a series of flight paths inside their roost, close to the exit, evaluating actively the environmental light conditions by the so called light-sampling behavior (Twente 1955; DeCoursey & DeCoursey 1964; McAney & Fairley 1988). The emergence behavior is also affected by age, reproductive status and body condition (Duvergé et al. 2000). To study the influence of such ecological parameters in the emergence and pre-emergence behavior I therefore monitored the emergence of the nursery colony of *R. ferrumequinum* near Pisa throughout the year, also focusing on the reproductive season when major changes occur in the pregnancy status of females and individuals of different ages (adults and juveniles) are present.

Despite the ability to use small roosts, even for the crevice dwelling bats to find a proper roost in a city is not an easy task. Also the populations of the more common species of bats are decreasing in urban and suburban habitats and the loss of roosts is one of the major cause of their decline (Agnelli et al. 2008). As cited above, the choice of a roost is influenced by some characteristics of the roost itself, such as internal temperature, and of the environment surrounding it (Fenton & Rautenbach 1986; Churcill 1991; Wunder & Carey 1996; Entwistle et al. 1997). Moreover, individuals usually shift between roosts and need multiple roosts throughout the year. However monitoring the presence of crevice dwelling bats in their standard roosts, both in urban and in more natural landscape, is difficult, due to the habit of these species to hide inside small crevices, in which are difficult to notice. Bat boxes, which are artificial roosts for bats, are usually used to monitor the presence of such species in a given area (Stebbings & Walsh 1991). These are also used as conservation tool to give bats an adequate roost, usually in forests, where a profit-oriented management often do not allow the presence of crevices in trees (Ciechanowski 2005). Also the colonization of such artificial roosts is influenced by the usual ecological features which affect the choice of roosts in buildings and natural crevices (White 2004). I therefore monitored some bat boxes installed in urban and suburban landscapes to study the ecological preferences for roosts by the human-tolerant crevice dwelling bats. I also focused on studying the influence of aspect of positioning and color of the roosts which directly influence the internal temperature of the roost (Laurenço & Palmeirim 2004). However, colonization rate of bat boxes is quite variable and often the majority of such artificial roosts result to be not colonized even years after their positioning (White 2004; Ciechanowski 2005; Flaquer et al. 2006; Lesinski et al. 2006). To raise the number of my sample of monitored roosts I decided to use a participatory approach to involve people in the monitoring.

One of the main benefits of using volunteers in monitoring is the inexpensiveness, which allows the collection of a large amount of data on different space and time scales with very low budget. However, an appropriate training of non-professionals by experts is needed (Newman et al. 2003). Generally volunteers have a lower efficiency compared to specialists and some tasks result unsuitable to be carried out by people with no experience, although with a proper training, they would potentially collect good quality data, improving their ability over time. More difficult tasks require a continuous training, while, for simple tasks, a single-event training may be enough (Newman et al. 2003; Goffredo et al. 2004). Selecting basic information to be collected by people may therefore be the key for a well participated monitoring program which would be able to gather viable data.

However, some topics are perceived as non-interesting by the general public, who is often more attracted to more appealing projects than to more useful projects, even in conservation biology (Beattie & Ehrlich 2010). Communication therefore plays a key role for the people involvement in a project which has "conserving bats" as the main aspect. It is therefore necessary to properly communicate a correct message, evaluating some aspects of the project that people can perceive as useful and close to them (Schultz 2011). Fortunately, bats are really useful organisms, they provide ecosystem services like insects control, pollination and seed dispersal and they are also good bio-indicators as they generally live in healthy environments (Dietz et al. 2009; Kunz et al. 2011). Through those aspect it is possible to capture the people interest, trying to involve them in a conservation project that also allow a correct spread of knowledge about its topic. This possible change in attitude represents the basis for a paradigm shift which may positively change people behavior toward bats (Schultz 2011).

Therefore the main topics that I intended to study during my doctorate were all related to the ecology, behavior and conservation of human-tolerant species of bats. More in particular I focused on:

1) Evaluating the influence of microclimatic and seasonal parameters on the roost use by bats in human artefacts, both in crevice dwelling species and in those that need large volumes;
2) Studying the emergence and pre-emergence behavior in a nursery colony of *R. ferrumequinum*, evaluating the influence of microclimatic, seasonal and demographic variables throughout the year;
3) Involving people in a participatory conservation project which is also focused on changing the attitude of the general public toward bats through raising knowledge and communication.

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2. Where and at what time? Multiple roost use and emergence time in greater horseshoe bats (*Rhinolophus ferrumequinum*)

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Where and at what time? Multiple roost use and emergence time in greater horseshoe bats (*Rhinolophus ferrumequinum*).
Under review in Acta Chiropterologica (2012)

2.1 Abstract

One of the causes of the decline in European bat populations is undoubtedly the gradual disappearance of their roosts, usually due to human interference, and even species which use human artifacts as roosts face this crisis. One of largest nursery colonies of *Rhinolophus ferrumequinum* in Italy inhabits the Natural Park of Migliarino San Rossore Massaciuccoli, using multiple roosts in buildings.

We identified the various roosts used by *R. ferrumequinum* within the park and investigated their microclimatic parameters (temperature, relative humidity and daily temperature excursion). We monitored roost use for one year and found it to be influenced by season and the microclimatic conditions of different roosts, but also by occasional stress conditions such as disturbance by predators.

We also studied nightly emergence behavior, specifically we considered how this is influenced by climatic (temperature, relative humidity and evening light intensity) and demographic (presence of pups, juveniles and colony size) parameters. We found that the onset of light-sampling behavior was anticipated at higher temperatures, lower evening light intensity, in larger colonies and in the presence of pups. High temperature and low evening light intensity resulted in a longer nightly emergence, which was also observed in larger colonies and in colonies with juveniles present.

This study highlights the importance of the conservation of multiple roosts within the distribution range of *R. ferrumequinum* nurseries. Additionally, we also demonstrated how certain climatic and demographic factors influence both light-sampling and emergence behavior.

KEY WORDS: *Rhinolophus ferrumequinum*, bats, ecology, emergence time, roost use, light-sampling.

2.2 Introduction

The greater horseshoe bat *Rhinolophus ferrumequinum* (Schreber 1774) is a Central Asian-European-Mediterranean species. It has been recorded in all regions of Italy, and typically favours areas below 800 m a.s.l. characterized by habitat mosaics (Agnelli et al. 2006). This species uses different roosts during different seasons throughout its range, and typically these roosts are separated by less than 30 km (Dietz et al. 2009). During winter, these bats utilize roosts in natural or artificial underground sites, with an internal temperature of 7-12 °C; while summer roosts are typically in artificial sites, such as buildings, caves and mines, and rarely in tree holes. During summer, males have solitary habits, while females congregate in large maternity colonies (20-200 adult females), often inside buildings in warm attics (Lanza & Agnelli 2002).

The diet of the greater horseshoe bat consists predominantly of moths and beetles, which they hunt after sunset (Duvergé & Jones 1994; Dietz et al. 2009). Insects peak in abundance at sunset (Racey & Swift 1985; Rydell et al. 1996), and the visual acuity of raptors, which may hunt on bats, decreases with brightness (Fox et al. 1976). Therefore, for most bats, emergence behavior at sunset is a tradeoff between the opportunity to hunt during the peak in daily activity of their prey and the risk of themselves being preyed upon by raptors (Fenton et al. 1994; Jones & Rydell 1994; Rydell & Speakman 1995; Speakman 1995). Nightly emergence in bats is principally regulated by an endogenous circadian rhythm (Erkert 1982), although climatic and ecologic conditions affect its onset and duration (Rydell et al. 1996; Shiel & Fairley 1999; Russo et al. 2007). Kunz and Anthony (1996) suggest, for example, that a higher temperature at sunset may favour an early nightly emergence due to the reduced intensity of torpor during the day. Physiologically, pregnancy seems to delay the emergence of females, encumbered by a heavy wing loading (Duvergé et al. 2000). Some species evaluate the appropriate light intensity at which to emerge with a light-sampling behavior (Twente 1955; DeCoursey & DeCoursey 1964; McAney & Fairley 1988). This pre-emergence behavior consists of a series of flight paths performed inside the roost close to the exit.

One of the largest maternity colonies of *R. ferrumequinum* in Italy inhabits the Natural Park of Migliarino San Rossore Massaciuccoli (PI). As females return from their winter hibernacula, typically mid-March, and occupy some buildings within the Park as roosts for the entire reproductive season (Agnelli & Guaita 2010). Pups are born in June and become fully independent after about 6 weeks. In horseshoe bats, as in other temperate zone bats, both the timing of birth and growth of pups are affected by climatic conditions (Ransome 1989; McOwat & Andrews 1995; Hoying & Kunz 1998; Ransome 1998; Kunz & Hood 2000; Hood et al. 2002; Reiter 2004; Dietz et al. 2007). In late autumn, individuals of this species which spent the summer in the Park migrate back to winter hibernacula, the location of which is still unknown (Agnelli & Guaita 2010).

In this study, in 2010 we monitored four different roosts used by the maternity colony of *R. ferrumequinum* that inhabit the Park . We also studied how microclimatic conditions and the presence of pups and juveniles may affect the onset of the light-sampling behavior of the maternity colony and the duration of the colonies' nightly emergence. Our hypothesis was that during their activity period (spring-autumn), bats require different roosts with different microclimatic features, in order to fulfil their various ecological needs during this period (Flanders & Jones 2009). Microclimatic conditions and seasons also directly affect the nightly emergence time of the colony (Kunz & Anthony 1996; Shiel & Fairley 1999; Russo et al. 2007). Additionally, the presence of pups causes early onset in light-sampling behavior and emergence of the maternity colony. This is likely caused by the higher trophic needs of females whilst lactating (Shiel & Fairley 1999; Duvergé et al. 2000; Russo et al. 2007;).

As pups grow they need to test their flight ability, generally repeatedly entering and leaving the roost, causing an increase in the duration of nightly emergence (Kunz & Anthony 1996).

2.3 Materials and methods

2.3.1 Study Area

The Natural Park of Migliarino San Rossore Massaciuccoli was established in 1979; it is located in Tuscany (Italy) and covers an area of approximately 230 km^2. We monitored the four buildings that the colony individuals use as roosts, mainly from March to October, in the Park:

- "Casematte" (CM): a former bunker transformed in 2007 during the LIFE ("L'Instrument Financier pour l'Environnement", the environmental funding program of the European Commission) project "Dunetosca" in an artificial underground site;
- "La Fagianaia" (LF): abandoned building historically used, since at least 2003, by the colony and fully renovated during the LIFE project "Dunetosca" to prevent collapse (2007/2008);
- "Il Forno" (IF): abandoned building occasionally used as a summer roost by the colony since 2007 (possibly earlier);
- "Cascine Nuove" (CN): complex of inhabited buildings with warm attics discovered to be in use as a roost during the present study.

CN was monitored only from May 2010. A map of the roosts is shown in Fig. 1, although we cannot publish their exact coordinates in order to avoid any disturbance by the general public.

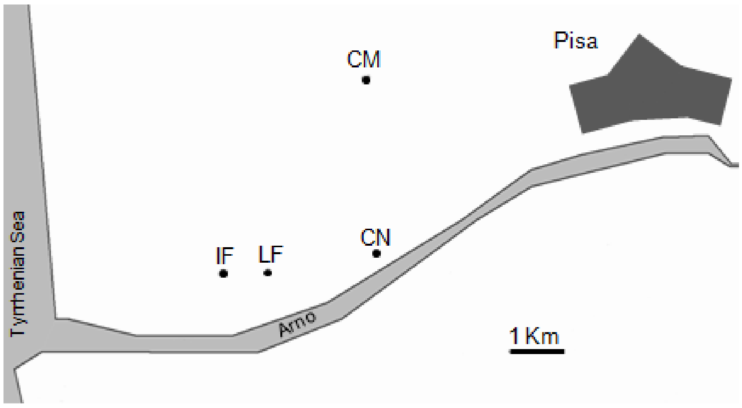

Fig. 1. Map of roosts used by the nursery colony of *R. ferrumequinum* in the Natural Park of Migliarino San Rossore Massaciuccoli (PI).

2.3.2 Monitoring methods

To monitor the microclimatic conditions of the first three roosts, we installed a sensor able to detect the internal conditions of temperature and relative humidity (HOBO Pro Series Temp, RH) at each roost between March and April. To monitor the external conditions, we placed a similar sensor on a wall outside the LF roost. We also placed a light sensor (HOBO TEMP, RH, LI, EXT) and a sensor to detect rainfall (HOBO Data Logging Rain Gauge - RG3) near the exit used by bats in LF. Temperature and relative humidity were recorded hourly, light intensity every five minutes and precipitation was recorded daily. The initiation and completion of monitoring in the various roosts are summarized in Table 1. Due to the late detection of CN in the study, we could not monitor the internal microclimatic condition of this roost. We counted the number of individuals in each roost every week, between March 24 2010 and March 18 2011, with the exception of CN where weekly counts commenced after its discovery on May 19 2010.

Table 1. Initiation and completion dates of the monitoring methods in the various roosts. External and internal sensors are also shown

Roost	Initiation	Completion
LF		
- T, Rh (internal)	April 2010	March 2011
- T, Rh (external)	April 2010	March 2011
- Light intensity (external)	March 2010	March 2011
- Rainfall (external)	March 2010	March 2011
CM		
- T, Rh (internal)	March 2010	March 2011
IF		
- T, Rh (internal)	March 2010	March 2011

On June 28 2010, after the birth of pups, we started to monitor the emergence of the nursery colony located in CN. The monitoring protocol was designed to minimize the disturbance towards the colony, thus we avoided catches and other methods which induce stress. We counted the individuals in a section of the attic, located in an intermediate position between the site where the colony rests and the available exits. The attic is partially illuminated by outdoor sunlight, due to the presence of some windows. The counting session started before sunset and ended when all of the individuals from the colony had left the attic or it was too dark to count. Given the location and the time chosen for the count, the animals were observed during light-sampling behavior. We performed the counts twice a week during July, in the presence of pups, and once a week for the rest of the study. During each session, we recorded the time at which the first bat performed light-sampling behavior, the end time of nightly emergence and the duration of emergence. We were able to retrieve data from the sensors associated with each roost concerning: temperature during nightly emergence, average daily temperature, relative humidity during nightly emergence, average daily relative humidity, evening light intensity (calculated from the average of the thirteen values recorded in the hour before sunset: Shiel & Fairley 1999) and mm of daily rainfall. To evaluate the exact time of sunset, we calculated the astronomical time of civil sunset (96°) for the nearby city of Pisa. After each counting session we inspected the room used by the colony as a roost to count pups, juveniles and adults still present, i.e. those which did not emerge from the roost. We considered "juveniles" to be individuals that appeared bigger then pups, with fur and potentially able to fly and still distinguishable from an adult. Monitoring of the nightly emergence of CN ended on October 7 2010.

2.3.3 Data Analysis

A two-way Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson 2001) design was used to test for differences in average temperature, relative humidity and daily temperature excursion recorded in each decade of each month in CM, LF, IF and outside. Resemblance matrix was based on Euclidean distance, and both factors, "season" and "roost", were fixed and orthogonal.

To evaluate the variation of the light-sampling with respect to the sunset, we calculated the anticipation (in minutes) of the onset of such behavior from the sunset time. Two one-way Permutational ANOVA designs were applied to analyze the influence of the month as a factor on (1) anticipation and (2) duration of nightly emergence, which was calculated from the onset of the light-sampling until the last output event from the roost. A Distance-Based General Linear Model (DIST-LM, Anderson et al. 2008) was utilized to study, separately, the relationships between the anticipation of the onset of light-sampling and (1) the climatic variables recorded externally (Table 1) and (2) the size of the maternity colony (measured on the basis of the adults leaving the roost) and the presence of pups and juveniles. We also used DIST-LM to evaluate how environmental and demographic parameters, separately, influenced the duration of the nightly emergence. We used the Akaike information criterion (AIC) as a measure of the relative goodness of fit of a statistical model. The alpha significance for PERMANOVA designs and for DIST-LM marginal tests was set to $p < 0.05$.

2.4 Results

2.4.1 Roost use

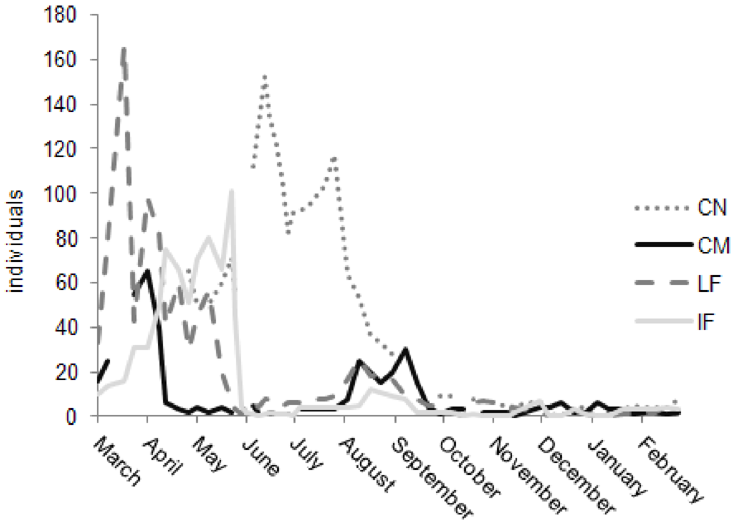

Fig. 2. Number of individuals counted in the various roosts during the 2010-2011 period. The delayed start in the CN series is due to a late individuation of the nursery, and the large gap in the series is the result of avoidance of the stressful count method at this time.

The presence of bats in roosts during the monitoring period is shown in Fig. 2. Use of CM as a roost was concentrated in April and September, with a maximum of 65 individuals counted on April 23; while in the remainder of the year, CM was used only occasionally by isolated individuals. LF was used as a roost by the colony from the beginning of spring, with a maximum of 166 individuals on April 9, and occupation decreased throughout the season until June, when no animals were recorded inside. During that period, we observed a green whip snake (*Hierophis viridiflavus*) in the courtyard of the roost and we recorded traces of a beech marten (*Martes foina*). From the end of August and throughout September a small number of individuals were recorded present in LF. Individuals in IF started to increase in abundance from mid April (31 individuals), coinciding with the gradual abandonment of LF, increasing to a maximum of 101 individuals on June 14. We then registered a sudden drop in abundance and the roost was abandoned before the end of June. During some nightly inspections in IF we found an ant colony (*Camponotus vagus*) foraging in the ceiling occupied during the day by bats, and on June 17 we observed five pups on the ground, alive but covered with ants.

Table 2. PERMANOVA test on differences in environmental factors across roost (ro) and season (se). All factors were treated as fixed and orthogonal. The degrees of freedom, df; Mean Squares, MS, value of the Pseudo-F statistic and its probability level, P, are shown.

Source	Df	MS	Pseudo-F	P
roost - ro	3	3077.4	83.486	0.0001
season - se	3	1832.2	49.705	0.0001
ro x se	9	286.37	77.688	0.0001
Res	128	36.861		
Total	143			

We monitored individuals in CN from May 19, counting about 65 individuals. Bat presence in this roost remained high throughout the summer, with a maximum of 152 individuals (adults, juveniles and pups) counted on July 5. The first pup was observed on June 14 and the first juvenile on July 5, 21 days later. During the survey of July 22 we counted no pups, 38 days after the discovery of the first pup. Subsequently bats then started to gradually leave CN, and at the start of October there were no more individuals inside the roost. We observed some isolated individuals in all four roosts during winter.

Microclimatic features strongly differed among roosts and seasons (Fig. 3; Table 2). Differences in roosts varied between seasons; post-hoc tests revealed that, climatically, LF and IF were not statistically different, whereas CM differed significantly from both LF and IF.

2.4.2 Emergence behavior

Month of the year influenced both the onset of light-sampling behavior (Pseudo-F: 10.87, $p < 0.01$, PERMANOVA) and the duration of nightly emergence (Pseudo-F: 6.04, $p < 0.01$, PERMANOVA).

The DIST-Linear Model approach revealed that average daily temperature (Pseudo-F = 26.36, $p < 0.01$, DIST-LM test), temperature during nightly emergence (Pseudo-F 18.18, $p < 0.01$, DIST-LM test) and evening light intensity (Pseudo-F 39.19, $p < 0.01$, DIST-LM test) significantly influenced the onset of light-sampling, when considered individually.

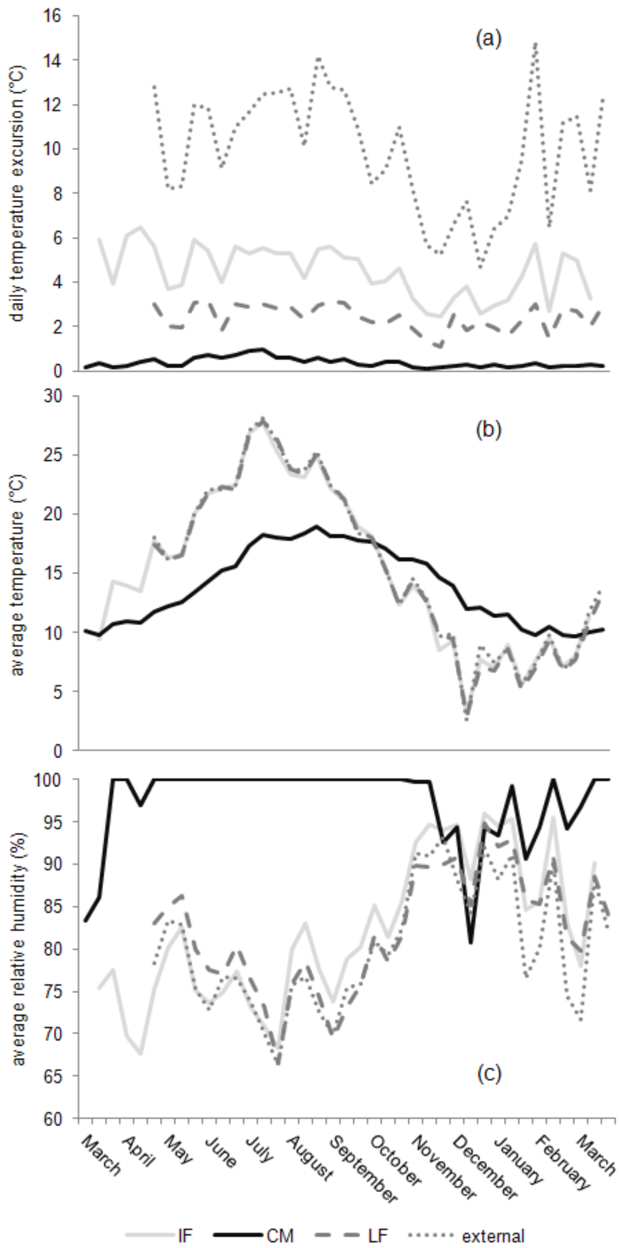

Fig. 3. Microclimatic features of roosts and external environment. (a) Daily temperature excursion; (b) average temperature; (c) average relative humidity.

The AIC model, that best described the variance of the data set considering the number of variables, was a combination of temperature during nightly emergence and evening light intensity (R^2: 0.88, DIST-LM test). The analysis of demographic parameters showed that colony size (Pseudo-F = 11.11, $p < 0.01$, DIST-LM test) and presence of pups (Pseudo-F = 12.85, $p < 0.01$, DIST-LM test), individually, significantly influenced the onset of light-sampling. The AIC model showed that the latter parameter alone explained approximately half of the variance (R^2: 0.45, DIST-LM test).

The environmental variables that individually significantly influenced the duration of emergence were, again, the average daily temperature (Pseudo-F = 22.30, $p < 0.01$, DIST-LM test), temperature during nightly emergence (Pseudo-F = 42.69, $p < 0.01$, DIST-LM test) and evening light intensity (Pseudo-F = 5.01, $p < 0.05$, DIST-LM test). The AIC model that best explained the variation in the duration of emergence was composed of the temperature during nightly emergence only (R^2: 0.78, DIST-LM test). The DIST-LM showed that, demographically, colony size (Pseudo-F = 5.51, $p < 0.05$, DIST-LM test) and presence of juveniles (Pseudo-F = 7.91, $p < 0.01$, DIST-LM test) also statistically influenced the duration of nightly emergence, while presence of juveniles alone best explained the variance in the data for the AIC model (R^2: 0.33, DIST-LM test).

2.5 Discussion

2.5.1 Roost use

This study demonstrates that greater horseshoe bats occupy different roosts even throughout the activity period (spring – autumn). In fact, choice of roost by bats depends on seasonal requirements and is largely influenced by microclimatic factors such as internal roost temperature and humidity (Fenton & Rautenbach 1986; Churcill 1991; Entwistle et al. 1997) and the surrounding environment (Wunder & Carey 1996). These requirements are related to the changing physiological needs of individuals across seasons (Dietz et al. 2009). Microclimatic features of LF and IF were broadly similar and statistically different from those of CM, which was reflected in the different use of those roosts throughout the seasons. Average climatic parameters of LF and IF displayed a similar trend to the external climatic parameters, with the exception of a lower daily temperature excursion (Fig. 3). These characteristics make these roosts particularly suitable for use by maternity colonies, as they require warm roosts to speed up the development of embryos during the period of pregnancy (Dietz et al. 2009). CM was characterized by a generally higher relative humidity, a low daily temperature excursion and a lower variability in average temperature throughout the year compared to the external environmental conditions. The features of these roosts are similar to underground sites that these bats use during the winter for hibernation (Dietz et al. 2009). However, CM was used for short periods during the spring and autumn as a transitional roost. These types of roost are used by individuals that migrate between summer and winter roosts, and they also allow a genetic exchange between different colonies meeting during migration (Flanders & Jones 2009).

Abandonment of LF and IF during the spring does not accord with observations made during previous monitoring (Agnelli & Guaita 2010). The presence of terrestrial predators of bats near the two roosts is likely the cause of this premature abandonment. Both green whip snakes and beech martens are good climbers, and the presence of some footholds inside the roosts may have allowed them to reach the bats hanging from the ceiling. The approach of these predators to the ceiling at night may cause a strong enough disturbance to result in the drop of pups left alone by their mothers. We did not observe any predatory act on the bats by these cited predators. However, using an IR surveillance camera, we verified the ability of the beech marten to use the footholds in LF to reach the ceiling. We can assume similar stress is caused by the ants in IF that, through direct attack, may cause the pups to drop. Thus, we confirm that the presence of non-flying predators with knowledge of the location of the roost, and the ability to reach the colony, can alone determine the abandonment of the roost (Agnelli & Guaita 2009).

2.5.2 Emergence behavior

The period of the year was found to be an important factor both for the onset of light-sampling behavior and the duration of the nightly emergence. This factor summarizes a set of climatic and ecological features and it is not surprising that emergence varies throughout the months; this also accords with the observations of Kunz and Anthony (1996).

Regarding the environmental parameters, we found temperature to significantly affect the onset of light-sampling behavior, with anticipation of this behavior at higher temperatures. This confirms the predictions of Kunz and Anthony (1996) concerning the anticipation of nightly awakening due to the lower intensity of torpor during the day. The ability of the temperate zone bats to assess the temperature outside the roost may, therefore, aid them in adjusting the moment of nightly awakening, correcting the endogenous rhythm, or more simply directly influencing the onset of the pre-emergence behavior. Evening light intensity was also found to significantly influence the onset of light-sampling. In particular, in dimmer conditions the onset of such pre-emergence behavior near the exit of the attic was anticipated, as was nightly emergence (Shiel & Fairley 1999; Duvergé et al. 2000).

Marginal tests showed that a larger colony favours an anticipation of the onset of the light-sampling compared to smaller colonies, in contrast to the findings of Avery (1986) for nightly emergence. Avery (1986) suggested that the delay he observed in the emergence for larger colonies may result in a social cost to individuals, specifically in terms of reduced hunting time. We can explain our results within the context of an assumption that a larger number of individuals leaving the roost may have an adaptive value in terms of anti-predatory response, both having a dilution and confusion effect during emergence, so the members of the colony can risk more and anticipate their leaving. We can also assume that a greater aggregation of animals facilitates the maintenance of a higher individual body temperature during the day, and this may favour an early awakening, as previously suggested for temperature. However, we found that the onset of light-sampling was mainly anticipated by the presence of pups inside the roosts. Previous studies have suggested that this also favours an early nightly emergence because of the greater trophic needs of lactating females, which have to find a compromise between the risk of being preyed upon and the need to hunt at times of high insect activity (Shiel & Fairley 1999; Duvergé et al. 2000; Russo et al. 2007). The same hypothesis can be proposed for the onset of light-sampling behavior. Our results accord with the observations of Duvergé et al. (2000) and contradict the predictions of Jones and Rydell (1994) about *R. ferrumequinum*. The authors argue that bats which feed predominantly on moths should be less sensitive to this hazardous anticipation of their emergence, as moths are more active during the night than at sunset. However, the diet shift of this species, which is focused on dung beetles in particular situations (Jones 1990; Jones et al. 1995), can explain the anticipation of the onset of both emergence and light-sampling of our colony, without contradicting the hypothesis of diet dependence in the emergence behavior of bats. The presence of a high proportion of landscape managed at pasture and a considerably large population of ungulates (Perfetti 2010), capable of supporting a rich community of coprophagus beetles, in the study area further validate our hypothesis.

Microclimatic parameters that individually influence the duration of the nightly emergence are the same as those which influence the onset of light-sampling. It is likely that these two emergence features are strongly correlated, and thus an anticipation of light-sampling may also cause a general extension in the duration of emergence. A high correlation coefficient between those features (0.80) seems to confirm this hypothesis. However, the model that best fits the variance of the data set does not include the evening light intensity.

Taken individually, colony size was found to have a statistically significant effect on duration of nightly emergence. In particular, in a larger colony the emergence is longer than in a smaller colony; this accords with the observations of Kunz and Anthony (1996) in multiple colonies of varying size. The authors also assumed that the extended emergence was indirectly due to the size of the colony. In fact, the increase in the number of individuals emerging at night, assessed during emergence surveys, occurred in conjunction with the weaning of juveniles, which may be the actual cause of the increased duration of the nightly emergence (McAney & Fairley 1988). Our data confirm this hypothesis, since the presence of juveniles inside the roost at night was the factor that best described the variance of the data set. Juveniles tend to leave the roost later than adults (Duvergé et al. 2000), and for approximately a week after they learn to fly they make prolonged flights within and outside the roost (Hughes et al. 1989), resulting in a longer duration of emergence. This particular behavior of juveniles is caused by the necessity to test and improve their ability to fly, mimicking in some way the light-sampling behavior. This "flight-sampling" behavior is performed near the roost exit, and at times with no risk of being preyed upon by raptors, probably due to the adaptive benefits, in terms of fitness, to young bats.

This study revealed some important features of the roost choice and emergence behavior of a well conserved Italian population of *R. ferrumequinum*. Knowledge concerning these populations is sparse, and thus their monitoring is important, particularly for conservation purposes. One of the most significant findings of this study was the importance of the presence of roosts with both similar and different microclimatic features. Similar roosts allow the colony to perform roost-switching in cases of disturbance by predators (including humans), while the existence of different roosts allows the presence of bats within the protected area throughout the majority of biological stages that these bats undergo during the year.

Acknowledgements. We thank the Corpo Forestale dello Stato personnel that work inside the Park, and dr. Antonio Perfetti and dr. Olga Mastroianni for their kind assistance. We also thank dr. Massimo del Guasta for technical support in the microclimatic sampling of the roosts. Many thanks to Giacomo Biasi for his precious help in the field.

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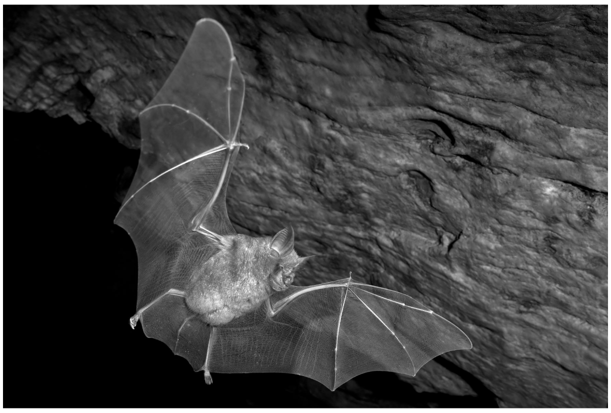
3. Emergence behavior in a nursery colony of *Rhinolophus ferrumequinum*



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In preparation

3.1 Abstract

The timing of nightly emergence at sunset in bats is a tradeoff between the opportunity to hunt during the peak in daily activity of insects and the risk of being preyed upon by raptorial birds. The onset of this behavior depends predominantly upon an endogenous circadian rhythm which is adjusted by a set of ecological variables.

We studied the influence of temperature, relative humidity, evening light intensity, precipitations, presence of pups and juveniles, and the size of the colony in shaping the characteristics of the emergence behavior throughout the year and in the reproductive season of one of the biggest Italian nursery colonies of *Rhinolophus ferrumequinum*.

During the year the onset of light-sampling was anticipated and the duration of emergence was longer with increasing temperatures and size of the colony. Focusing on the reproductive season, we found that the onset, mean and median of nightly emergence were anticipated in evenings with dimmer condition while the presence of juveniles caused the duration to be longer. Distribution of intensity of the emergence tended to be not normal both in presence of pups and when the number of individuals emerging were higher than 100.

Environmental temperature and larger colonies likely allow a passive facilitated rewarm at evening which caused an earlier onset of the light sampling. The influence of evening light intensity is likely linked to the decreased predation ability of raptorial birds in dimmer conditions, which allow adult bats to emerge earlier. The avoidance of the predation risk explained also the later emergence of juveniles which have a low flight ability, causing a disruption in the approximately normal distribution of intensity of such behavior.
KEY WORDS: *Rhinolophus ferrumequinum*, bats, ecology, emergence time, light-sampling.

3.2 Introduction

Choosing the proper timing for the nightly emergence at sunset is vital for bats. Every evening they need to find a compromise between the opportunity to prey and the risk of being a prey (Fenton et al. 1994; Jones & Rydell 1994; Rydell & Speakman 1995; Speakman 1995). In fact, leaving the roost earlier in the evening exposes bats to an higher risk of being caught by raptorial birds which may still be active. On the other hand, emerging later, bats risk to miss the moment of higher insect activity during the day (Racey & Swift 1985; Rydell et al. 1996), although visual acuity of diurnal predators decreases rapidly

(Fox et al. 1976). Thus, the ability of shifting the time of nightly emergence is the adaptive answer to the evolutionary tradeoff of eating without been eaten.

The basis for the adjustment of the timing of nightly emergence are mainly regulated in bats by an endogenous circadian rhythm (Erkert 1982). Further ecological information, which bats collect directly or indirectly by the environment, are additionally used to fine tune the proper timing of emergence from the roost. The evening light intensity influence the hunting ability of raptorial birds which are less efficient when light is low. Evaluating light intensity therefore allow bats to emerge earlier with respect to the sunset in dimmer conditions (Shiel & Fairley 1999; Duvergé et al. 2000; Russo et al. 2007). Some bat species roost in crevices open to the external where they indirectly evaluate the environmental light intensity, while other species roost in closed shelters where external light do not enter. These latter species often perform a series of flight paths inside their roost, close to the exit, to evaluate actively the environmental light conditions in the so called light-sampling behavior (Twente 1955; DeCoursey & DeCoursey 1964; McAney & Fairley 1988). Higher temperature, either caused by an higher environmental temperature or by the vicinity of many other individuals in bats colonies, facilitate the evening arousal (Kunz & Anthony 1996), thus anticipating the onset of the light-sampling (see Chapter 2). Even environmental relative humidity appears to have some influence in the nightly emergence (Shiel & Fairley 1999).

The onset and distribution of intensity of the emergence behavior is also affected by age, reproductive status and body condition (Duvergé et al. 2000). Under energetic stress due to low body reserves, bats tend to emerge earlier, taking more risk of being preyed. Lactating females in nursery colonies, for example, leave their roost earlier in the evening, due to the higher energy demand of lactation, while pregnant females tend to leave later due to a heavier wing loading which encumber their flight capacity (Duvergé et al. 2000). The flight ability also affect the emergence of young bats, which, after the weaning, need to improve their capability. To avoid the risk of predation, juvenile bats tend to emerge later than adults, causing an higher duration in the nightly emergence of the colony (Kunz & Anthony 1996; Duvergé et al. 2000; see Chapter 2).

We studied the emergence behavior of a nursery colony of *Rhinolophus ferrumequinum* which live in the Natural Park of Migliarino San Rossore Massaciuccoli near the city of Pisa in Tuscany (Italy). This bat species feed predominantly after sunset on moths and beetles (Duvergé & Jones 1994; Dietz et al. 2009). The necessity of an anticipation of the nightly emergence for this species has been debated by some studies due to the diet which is mainly oriented on insects active at night (Jones & Rydell 1994). However Duvergé et al. (2000) found that the reproductive status in females influenced the emergence behavior of this species, anticipating during lactation and postponing during pregnancy, and Maltagliati et al. (see Chapter 2) found that also temperature and evening light intensity influenced the onset of the light-sampling behavior during the reproductive season. This discrepancy may be due to a diet shift which is reported in some nursery colonies of this species during some periods of the year and under particular circumstances (Jones 1990; Jones et al. 1995). We therefore studied the main features of the emergence behavior, both throughout the year and in the reproductive season only, in order to evaluate the influence of potentially significant microclimatic and demographic parameters on the timing and on the distribution of intensity of the nightly emergence.

3.3 Materials and methods

The nursery colony of *R. ferrumequinum* that we studied is the largest among the three known in Tuscany. It roosts predominantly in an attic of a building in the Natural Park of Migliarino San Rossore Massaciuccoli (see Chapter 2) as summer shelter, while its winter hibernacula are still unknown (Agnelli & Guaita 2010). We monitored the nightly emergence of the colony since 8 April 2011 to 12 April 2012 counting the individuals which were emerging from the roost. The counts were performed outside the building, close to the only exit from the attic, to not disturb the emergence behavior. They started before the sunset and during each session we recorded the time at which the first bat left the roost and then we recorded the number of bats emerging in each subsequent five minute period. The counts ended when no bats left the roost for ten minutes consecutively (Battersby 2010). After each counting session we inspected the attic to count pups, juveniles and adults still present. Juveniles appeared bigger than pups but smaller than adults, with little grey fur and potentially able to fly. We thus collected demographic data about adults emerged from the roost (adults), presence of pups (pups) and presence of juveniles (juveniles) inside the roost. We also noted the time at which the first bat performed light-sampling behavior inside the building (see Chapter 2) and we evaluated the exact time of sunset, calculating the astronomical time of civil sunset for the nearby city of Pisa which is five kilometers far. We performed the counts twice a week in the reproductive season since 9 June to 4 August 2011 and weekly in the rest of the period.

To collect microclimatic data about the roost we installed a sensor able to detect the internal conditions of temperature and relative humidity (HOBO Pro Series Temp, RH) inside the attic. To monitor the external conditions, we placed a similar sensor on a wall outside a close building. A light sensor (HOBO TEMP, RH, LI, EXT) and a sensor to detect rainfall (HOBO Data Logging Rain Gauge - RG3) were also deployed at the same site. Temperature and relative humidity were recorded hourly, light intensity every five minutes and amount of precipitations was recorded daily. Through those sensors, we retrieved data about average daily temperature (adt), temperature during the onset of nightly emergence (tde), average daily relative humidity (adrh) and relative humidity during the onset of nightly emergence (rhde) both for the roost (R-) and for the external (E-). We also retrieved data about evening light intensity (elt, calculated from the average of the thirteen values recorded in the hour before sunset: Shiel and Fairley 1999) and mm of daily rainfall (rain).

To study the nightly emergence we calculated five different features describing such behavior: first bat emerged (FBE), first bat performed light-sampling (FBLS), mean of emergence (mean), median of emergence (median) and duration of emergence (duration). We calculated FBE and FBLS as an anticipation to the sunset subtracting from the time of sunset the time of first bat leaving the roost and the time of first bat performing the light-sampling behavior, respectively. Mean and median were calculated evaluating the delay of each individual from FBE. Duration was calculated subtracting from the time of the last bat leaving the roost the time of the first. Prior to the analysis we studied the correlations between the parameters with a Draftman plot correlation table to select only those correlated less than 0.85. A Distance-Based General Linear Model (DIST-LM, Anderson et al. 2008) was utilized to study, separately, the relationships between those features and the (1) microclimatic and the (2) demographic variables. We used the Akaike information criterion (AIC) as a measure of the relative goodness of fit of a statistical model. We repeated those analysis also for the period in which the roost is used by at least 20 individuals (since 8 April to 29 September 2011) to study the emergence also during the actual presence of the nursery colony in the reproductive season.

For each count we also evaluated the normality of the distribution of the emergence through a Jarque-Bera test (Jarque & Bera 1987) which is based on the skewness and the kurtosis of the distribution curve. We then considered the normality or not-normality of the emergence distribution as a binary factor to compare separately both microclimatic and demographic variables with a two-way Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson 2001). Resemblance matrix was based on Euclidean distance. We also compared the normality of the distributions with a contingency table, using some demographic variables to discriminate our data: presence of pups; presence of juveniles; individual leaving > 50; individual leaving > 100. We considered only the counts with 5 or more individual emerging and Monte Carlo significance was preferred due to the low number of events of some emergence counts.

3.4 Results

Bats emerged during all the monitoring period except in 3 November 2011 and in the 9 December 2011 – 21 February 2012 period. However the number of bats leaving and dwelling inside the roost was extremely low (< 10) in between 13 October 2011 and 20 March 2012. The number of individuals emerging varied from 1 to 150. The first pup was recorded on the 9th of June while the last one on the 7th of July 2011. The first individual recognized as a juvenile was recorded on the 23rd of June while the last one on the 28th of July 2011 (Fig. 1a).

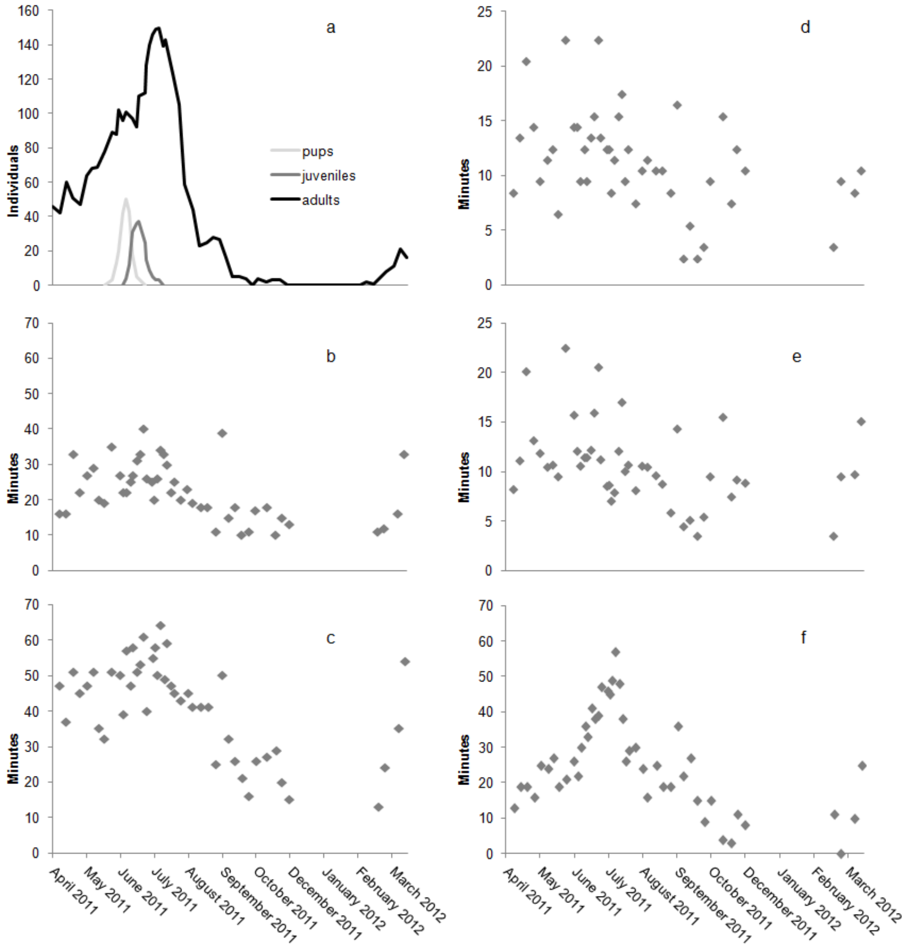

Fig. 1. (a) Number of adults emerged from the roost and pups and juveniles found inside it after the emergence of the adults; (b) Timing of first bat emerged (FBE); (c) Timing of first bat performing light-sampling (FBLS); (d) Median of the emergence behavior (median); (e) Mean of the emergence behavior (mean); (f) Duration of the emergence behavior (duration).

The average daily temperature at roost (R-adt vs R-tde: 0.99; R-adt vs E-adt: 0.98; R-adt vs E-tde: 0.93), the external average daily temperature (E-adt vs E-tde: 0.96; E-adt vs R-tde: 0.96), the temperature during emergence at roost (R-tde vs E-tde: 0.94) and the external temperature during emergence resulted to be highly correlated between each other. Also the average daily relative humidity and the relative humidity during emergence inside the roost were highly correlated (0.95). Therefore we selected the average daily temperature inside the roost as representative for the first set of parameters and the average daily relative humidity inside the roost for the latter.

Most of the AIC models that best described the emergence features for the entire year had low R^2 value considering the number of variables (Table 1). The only exceptions were the model for the microclimatic parameters of FBLS and those for the duration. Concerning FBLS, the AIC model showed that the average daily temperature explained about a quarter of the variance by itself (0.26) and the size of the colony was also important (0.43). In particular the individuals anticipated the light-sampling behavior when temperature or size of colony raised. The duration is explained quite well by the average daily temperature (0.45) and by the size of the colony (0.60). When those variables were higher the duration increased.

Table 1. AIC models generated by the DIST-LM analysis for the study of the emergence behavior throughout the year. Microclimatic and demographic variables are considered separately

Emergence features	Tot. variables*	Best model	AIC	R^2
FBE				
microclimatic	6 (0.16)	E-rhde, R-adrh, rain	170.36	0.35
demographic	3 (0.33)	adults	173.47	0.23
FBLS				
microclimatic	6 (0.16)	R-adt	210.72	0.26
demographic	3 (0.33)	adults	199.33	0.43
Mean				
microclimatic	6 (0.16)	rain	115.28	0.21
demographic	3 (0.33)	juveniles	121.97	0.07
Median				
microclimatic	6 (0.16)	elt	122.06	0.21
demographic	3 (0.33)	juveniles	126.44	0.13
Duration				
microclimatic	6 (0.16)	R-adt	198.48	0.45
demographic	3 (0.33)	adults	184.69	0.60

*Inside the brackets is given the expected value of R^2 for each variable, considering their total number

Considering the reproductive season, when a higher number of individuals used the roost, AIC models described better some other emergence features according to the R^2 values (Table 2). Concerning FBE, mean and median, the AIC showed that elt was the variable that described better their variance (respectively 0.47, 0.48, 0.53) explaining about the half. In particular, individuals generally anticipated their emergence when the evening light intensity were lower. The presence of juveniles was the variable that explained better the duration of the emergence in the reproductive season (0.46), increasing the duration when juveniles were more.

Table 2. AIC models generated by the DIST-LM analysis for the study of the emergence behavior during the reproductive season. Microclimatic and demographic variables are considered separately

Emergence features	Tot. variables*	Best model	AIC	R^2
FBE				
microclimatic	6 (0.16)	elt	101.12	0.47
demographic	3 (0.33)	juveniles	115.67	0.14
FBLS				
microclimatic	6 (0.16)	elt	127.35	0.21
demographic	3 (0.33)	adults	124.31	0.29
Mean				
microclimatic	6 (0.16)	elt	69.17	0.48
demographic	3 (0.33)	juveniles	86.84	0.06
Median				
microclimatic	6 (0.16)	elt	69.66	0.53
demographic	3 (0.33)	juveniles	89.00	0.11
Duration				
microclimatic	6 (0.16)	E-adrh, R-adt, R-adrh, elt	143.96	0.27
demographic	3 (0.33)	juveniles	129.15	0.46

*Inside the brackets is given the expected value of R^2 for each variable, considering their total number

Jarque-Bera test showed that 17 out of the 41 counts of nightly emergence actually performed by 5 or more individuals were normally distributed. PERMANOVA did not find any evidence about the influence of microclimatic (PseudoF = 0.52, $p > 0.05$) or demographic (PseudoF = 1.87, $p > 0.05$) parameters on the normality of distribution of the emergence behavior. Contingency table found that the presence of juveniles ($\chi^2 = 5.66$, $p < 0.05$) and a number of individuals emerging higher than 100 ($\chi^2 = 4.47$, $p < 0.05$) significantly explained the not-normality of the distribution of the nightly emergence. Moreover, the presence of pups ($\chi^2 = 0.17$, $p > 0.05$) and a number of individuals emerging higher than 50 ($\chi^2 = 0.58$, $p > 0.05$) did not significantly influence those statistical distributions.

3.5 Discussion

According to previous monitoring the roost was used by the nursery colony mainly during the reproductive season (see Chapter 2). However some individuals used the roost also during late autumn and winter, before migrating to hibernacula. Few individuals were recorded inside the roost even when the rest of the colony left the study area to hibernate in winter roosts. It is likely that these few individuals were inexperienced young bats which did not know how to reach hibernacula and failed to follow the rest of the colony. They still emerged, although less frequently, during winter, sometimes switching their roost. Bats, in fact, arouse and leave their roosts during winter when temperature were high enough to have some chance of feeding on insects (Avery 1985; Hope & Jones 2012) and to rehydrate (Thomas & Cloutier 1992).

The analysis of the emergence behavior showed that the parameters influencing it are not the same along the whole year and within the reproductive season . Concerning the entire season, bats tended to arouse earlier anticipating their light-sampling behavior when the size of the colony was larger. This confirms the prediction of Kunz and Anthony (1996) that suggested that larger number of individuals roosting together allow them to maintain a higher body temperature thus facilitating the arousal, which is the most energetically expensive phase of the torpor strategy (Prothero & Jurgens 1986). This energy saving strategy hypothesis is confirmed also by the fact that even higher temperatures inside the roost influenced the arousal, causing bats to anticipate the onset of such behavior. Temperature and size of colony also influenced the duration of the emergence, confirming what was found in previous monitoring (see Chapter 2). The increase of the duration, in larger colonies, was also observed by Kunz and Anthony (1996) who, however, suggested that colony size influenced the emergence only indirectly, hypothesizing that the actual cause of such variation was the presence of juveniles (McAney & Fairley 1988). We did not found any evidence of such influence, at least in the analysis of the emergence throughout the year. Even the temperature likely influenced the duration of emergence just indirectly, in fact during the reproductive season, when the colony is larger, also the temperature resulted higher due to the season. The close relationship between these variables was also confirmed by their correlation coefficient (0.73).

Focusing on the reproductive season, the most important environmental variable for the emergence resulted to be the evening light intensity which caused an anticipation of such behavior. The first bat emerged, and the mean and median of the distribution of the emergence resulted highly influenced by this parameter. The fact that, in dimmer condition, bats emerged generally earlier confirms that bats adopt an anti-predatory behavior to avoid the risk of being caught by avian predators (Duvergé et al. 2000). However this parameter resulted less important in the analysis of nightly emergence throughout the year. This discrepancy may be due to the higher energetic demand of lactating females in the reproductive season (Duvergé et al. 2000). When lactating, in fact, females need to feed their pups too and therefore the tradeoff between the risk of being preyed upon and the necessity to hunt at times of high insect activity (Shiel & Fairley 1999; Duvergé et al. 2000; Russo et al. 2007) becomes even more vital. Jones and Rydell (1994) argued that *R. ferrumequinum*, which is a bat that feed predominantly on moths, should be less sensitive to the necessity of an anticipation of the emergence, as moths are more active during the night than at sunset. However in particular situations this species shift its diet, focusing mainly on dung beetles (Jones 1990; Jones et al. 1995). The study area is actually characterized by the presence of a high proportion of landscape managed at pasture and a considerably large population of ungulates (Perfetti 2010) which can support a rich community of coprophagus beetles. The shift in diet may therefore explain why even *R. ferrumequinum* needs to face the compromise between predation and being a prey. Further study in the actual diet of the population of this species in Migliarino, San Rossore, Massaciuccoli throughout the year may help to clarify this point. We also found that, according to prediction of Kunz and Anthony (1996), the presence of juveniles influenced directly the duration of the emergence behavior. In fact when they become able to fly, young bats tend to leave the roost after the adults and, in the first week, they perform prolonged flights within and outside the roost (Hughes et al. 1989). This anti-predatory behavior, similar to the light-sampling of adults, is used to test and improve their ability to fly and cause a longer duration of the emergence. (Duvergé et al. 2000).

The nightly emergence in *R. ferrumequinum* was normally distributed during some events but the normality was not a general rule. Contingency table showed that for a number of bats emerging higher than 100 and in presence of juveniles, the distribution of the emergence of nursery colonies tended to be not normal. Nevertheless it is likely that the size of colony influenced this feature only indirectly while the juveniles are the actual cause of the not normality of the distribution. The presence of such individuals coincided in fact with the higher number of individuals emerging from the roost. As we discussed above, juveniles tend to leave the roost later in the night, after most of adults already left. This generated a bimodal shape of the distribution of nightly emergence, with a small temporal gap between the peak of adults emerging and those of the juveniles.

Our study highlighted some important features of the nightly emergence behavior of *R. ferrumequinum* which was influenced both by microclimatic and demographic parameters in nursery colonies. We showed that this species likely adjust the timing of arousal according to an energy saving strategy depending on temperature and size of the colony. We also confirmed the influence of evening light intensity on the onset of the nightly emergence as an anti-predatory behavior in the reproductive season even for a species which predominantly feeds on moths later on night. This may be linked to a diet shift which may be caused by environmental (i.e. abundance of insects over pasture) or physiological conditions (i.e. lactating) that should be investigated more in details. Finally we evaluated the importance of juveniles, which emerge later in the night with respect to adults to avoid the risk of predation, thus disrupting the approximately normal distribution of intensity of the colony as whole.

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4. Artificial roosts for bats: education and research. The “Be a bat’s friend” project of the Natural History Museum of the University of Florence

Paolo Agnelli, Giacomo Maltagliati, Laura Ducci, Stefano Cannicci.
Artificial roosts for bats: education and research. The “Be a bat’s friend” project of the Natural History Museum of the University of Florence.
Published in Hystrix (2011) 22, 215-223

4.1 Abstract

The project “BAT BOX: Be a bat’s friend” was launched in 2006 with the aim of spreading correct information about the ecological role of bats, rehabilitating their image and fostering the involvement of the public in conservation actions.

A wooden, single-chamber bat box was designed and produced at low cost. Through collaboration with Coop, to date ca. 25000 bat boxes have been sold over much of the country at cost price. Private citizens, institutions and associations installed and monitored the boxes using a standard form for the collection of data. The sale of a range of project-related merchandising articles has raised funds used for bat research and conservation projects. Conferences, public meetings, school lessons, papers and an informative brochure helped to spread the aims of the project. In 2010, Disney Italia produced a new brochure and the educational comic strip “Donald Duck and Kiro the Bat”.

The monitoring of bat boxes showed a progressive increase in the success of colonization over time, up to 40.0%. In general, both time from installation and height above ground proved to significantly influence colonization success.
KEY WORDS: Chiroptera, conservation, bat box, Italy

4.2 Introduction

The decline of bats all over Europe (Stebbings 1988; Hutson et al. 2001; Bontadina et al. 2008) is a matter of widespread concern. In Italy, in the last decades several large bat colonies have declined or disappeared (Agnelli 2006). Currently 14 species are listed as “threatened” (CR, EN, VU) and 5 as “near threatened” in the National Red List for Mammals, while data are insufficient to assess the status of a further 5 species (DD) (GIRC 2007).

Today the main challenge facing European chiropterologists is the need to intervene with concrete conservation initiatives for the protection of all bat species.

Prejudices and superstitions still play a role in the relationship between bats and human beings, so the circulation of correct scientific information is among the major actions to be taken for the conservation of bats

In 2006, in collaboration with the Town Council of Fiesole (province of Florence, Tuscany), during a public meeting, 10 artificial roosts (bat boxes) were distributed free of charge, while many people decided to build their own on the basis of supplied construction diagrams. Following the enthusiastic participation of the public and the rapid colonisation of some bat boxes, we decided to map out a more complex and extensive information project to involve a broader public.

The project “BAT BOX: Un pipistrello per amico” (Be a bat’s friend) was launched in 2006 with the aim of informing people about I) the life of bats, the importance of their ecological role and their usefulness for the control of insect populations, II) the underlying biological reasons and potential benefits for human welfare of a conservation program and III) the importance of peoples’ involvement for the success of the program itself.

To realize this goal, essentially four things were necessary:

- a model of a bat box that was effective while simple and cheap;
- mass production, to enable the construction of a large number of bat boxes at moderate cost;
- good distribution capacity, so as to make the bat boxes available over a large area;
- the ability to advertise the project adequately so as to reach a large number of people.

4.3 Materials and methods

To design the bat box, some available models were checked and assessed (Stebbings & Walsh 1991; Mitchell-Jones & McLeish 1999; Tuttle et al. 2005). The resulting model (Fig. 1) was made entirely of wood, with a single internal chamber and one entrance at the base. The details of the construction techniques were further refined with the three different firms that have taken over from each other in the construction of our bat boxes over the last four years.

A collaboration agreement was drawn up with Unicoop Firenze, a retail distribution chain with several sales outlets scattered over most of Tuscany. In 2007, the collaboration with Coop led to the construction of about 220 bat boxes, which were distributed free of charge to various Town Councils, mainly in Tuscany, which, in their turn, offered them to volunteers. To satisfy the large number of requests, in 2008 bat boxes were directly sold through the Coop supermarket chain. A BAT BOX logo (registered trademark) was produced with the graphic designers of Unicoop Firenze, to provide all the material related to the project with a characteristic graphic symbol. Stimulated by the public success achieved in Tuscany, Unicoop Tirreno and Coop Adriatica in 2009 and Coop Lombardia, Coop Estense and Coop Consumatori-NordEst in 2010 offered their collaboration to the project. As a consequence, we formalised the liaison directly with Coop Italia, which co-ordinates all the Italian cooperatives, ensuring the spread of the project over most of the country, from Campania, in the south, to Lombardy and Friuli Venezia-Giulia, in the north. This collaboration offered a wide array of benefits:

- the availability of numerous firms - linked to the Coop by long-standing commercial relations - for the standardised and economic construction of the bat boxes;

- distribution and sale of the bat boxes over most of the country through a broadly ramified network;

- the possibility of utilizing the advertising channels of Coop to promote the product to its members;

- the possibility of selling the bat boxes to the public at the production cost;

- the vast potential for creating and selling merchandising material connected with the project to raise funds for the project itself and research on Chiroptera in general.

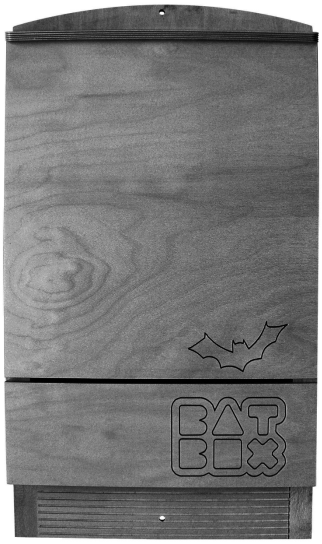

Fig. 1. Bat box designed by the Natural History Museum of Florence

The efficiency of bat boxes in attracting bats was monitored by volunteer collaborators through a specific form for the collection of data about the features of the location, modes of installation and results of regular checks for the presence of bats and or faeces (Table 1).

Boxes were checked at least once a month and considered as used by bats whenever an individual or faeces were detected at least once. During the monitoring of the bat boxes the species were not identified by the volunteers in order to avoid disturbance and the consequent risk of the roost being abandoned. To identify which factors had the greatest influence on the success of colonisation, we carried out a multivariate analysis, using PERMANOVA

(Permutational Anova), while a SIMPER (Similarity percentages) test was used to appraise the impact of the factors identified.

Table 1. Data of installation and monitoring of the bat boxes

Owner bat box	Name, address, e-mail, telephone.
Installation data	Date of placement
	Height from the ground
	Height from the floor
	Orientation
	Hours of sunlight
	Date of purchase
	Distance from water bodies with a diameter of at least 1.5 m (classes)
	Distance from tree-lined squares, parks, forests (classes)
	Placed on: building/tree/pole/other
Monitoring data	Number of specimens and of excrement (checked every 10 days)
	Notes

To foster the exchange of data between the volunteers and our working group, we then set up an e-mail address for the project, batbox@unifi.it, while to enhance involvement and information, several web pages (www.msn.unifi.it) were created to spread the aims and results of the project, together with information on bats and the downloadable leaflet "Un pipistrello per amico" ("Be a bat's friend"; Agnelli et al. 2007; 2009). The latter, periodically updated since 2007, is also delivered together with the bat box and distributed in the Coop supermarkets. During the first 5 years of activity information on bat ecology was spread through conferences and public meetings, in liaison with Town Councils, private associations, the teaching staff of primary, middle and secondary schools and the technicians from local health agencies (ASL).

Moreover, we released TV interviews and informative papers were monthly published on the magazine "L'Informatore", published by Unicoop Firenze in about 650000 copies, and, opportunistically, on other magazines and newspapers. Finally, the graphic designers of the Museum "La Specola", Florence, and Coop have collaborated in the design of many gadgets on naturalistic themes, including t-shirts, beach towels, exercise books, pencil cases, satchels and school diaries, all accompanied by our logo and a brief description of the project.

4.4 Results

The number of bat boxes sold every year grew in an almost exponential manner, from 3000 bat boxes sold in 2008 up to 8000 in 2009 and over 14000 in 2010 (between the months of March and August alone). To this total of about 25000 bat boxes sold at cost price we have to add thousands of bat boxes which, over the last two years, have been sold by other firms, both retail and online, or built by private citizens.

So far the advertising campaign has included:

- 42 public meetings (conferences, bat-nights and lessons) held by our working group;

- 112 papers published in both local and national newspapers and magazines (www.msn.unifi.it/CMpro-v-p-938.html);

- 10 TV interviews produced and broadcast on local and national channels, even involving a Swiss TV channel (www.msn.unifi.it/CMpro-v-p-1078.html);

- over 5000 e-mails sent to batbox@unifi.it

- the sale of approximately 41694 t-shirts, 8651 beach towels and about 138318 articles of school stationery (www.msn.unifi.it/CMpro-v-p-933.html);

- Walt Disney Italia took over for us the graphic design of the new guide to bats and the bat box, entitled “Un pipistrello in famiglia” (A bat in our family; Agnelli et al. 2010) and further supported our project through the creation of an original comic story “Paperino e il pipistrello Kiro” (Donald Duck and Kiro the Bat), who lead young readers through the world of bats (Fig. 2).

A total of ca. € 100000 has been gathered so far to finance bat research, allowing also the funding of a three-year PhD and the payment of professional collaboration fees to young graduates.

At present (2010), we have received about one thousand registration forms from people engaged in the study, but only a small percentage of bat-boxes (about 30%) has been checked once a month and so considered suitable for statistical analyses. In 2007 we recorded a colonisation success of 20.6% (N= 68). In 2008, colonisation success was 34.9% (N= 43) for the boxes set up the previous year and 16.0% (N= 81) for the boxes set in position and correctly monitored in 2008.

In 2009, colonization success rose to 40.0% (N= 30) and 25.5% (N= 51) for the boxes set up in 2007 and 2008, respectively, while that of the boxes installed in 2009 was 11.5% (N= 61).

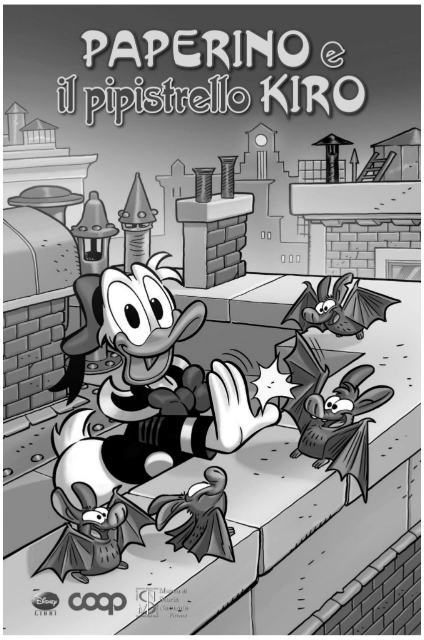

Fig. 2. Disney comic booklet “Donald Duck and Kiro the Bat”

Multivariate analysis showed a significant difference between colonised and non-colonized bat boxes (Pseudo F: 4.94, $p < 0.01$). The variables height from the ground, months of placing in position and hours of exposure of the bat-boxes to sunlight explained 66.32% of the variability of the data (24.15%, 23.77% and 18.39%, respectively).

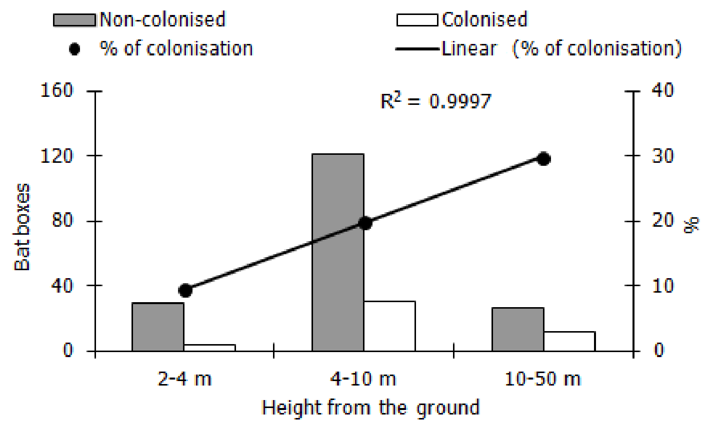

Fig. 3. Univariate analysis of the variable “height from the ground”

The success of colonization increased linearly with the height from the ground at which the bat box was positioned (R^2: 0.9997; Fig. 3), while the trend in terms of hours of exposure to sunlight was unclear (Fig. 4). Colonization success clearly increased (R^2: 0.9194) with the time that bat boxes were in place (Fig. 5).

In 2008 colonization increased (R^2: 0.9201) throughout the monitoring season with a peak in October, while in 2009 the peak of bat occurrence into bat boxes was in August, two months earlier than the previous year.

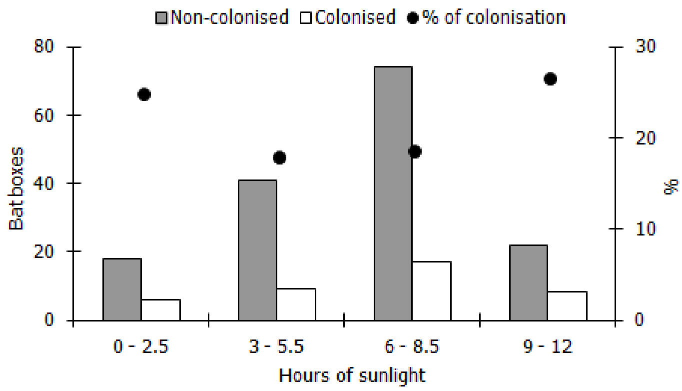

Fig. 4. Univariate analysis of the variable "hours of sunlight exposure"

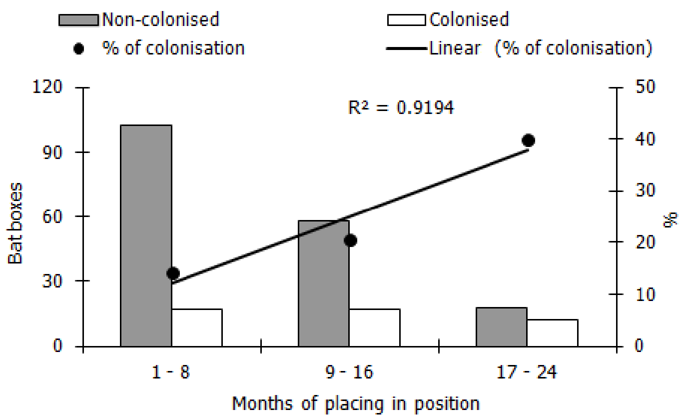

Fig. 5. Univariate analysis of the variable "months of placing in position"

4.5 Discussion

The educational and informative campaign “BAT BOX: Un pipistrello per amico” spread over a vast area of Italy. Considering its features of popularity and continuity, it is undoubtedly the largest awareness-raising campaign carried out in Italy in favour of bats, and ranks among the most important European initiatives. As a result of the enthusiastic participation of so many people who have contributed to the growth of the project, we hope that the need for bat conservation has come to be shared by a large part of the population.

As regards the experimentation of the bat boxes, bats’ preference for high box-sites can be interpreted as the search for safer roosts, while the increase of bat colonization with time depends on both the increasing probability of discovery and reliability of the bat boxes which have been placed in position for longer . The absence of a clear trend with respect to the hours of sunlight could perhaps be explained by the different microclimatic requirements of the two sexes: males generally tend to prefer cooler roosts, since these are better suited to daytime rest in a state of torpor, while mature females need warmer quarters for reproduction (Lanza 1959). Finally, the increase in the number of colonization between late summer and autumn suggests that boxes are mainly occupied by young bats and adults for autumn mating. Ongoing monitoring will enable us to analyse the results over a larger number of seasons and point out more accurately which parameters dictate the success of our bat boxes. A future implementation of the project will involve faecal analysis to confirm the effectiveness of bats in pest control.

Scheduled for 2011 is the creation of two new models of bat box - one in wood and a second model made of inert material and recycled plastic - which are innovative, cheaper, and hopefully as efficient as the previous model. A new information campaign will be launched with the support of Coop and Disney’s Kiro.

Acknowledgements. Our most sincere thanks go to Maura Latini, Roberta Corridori and Antonio Comerci (Coop) who contributed both their expertise in the areas of advertising and management and their passion and social commitment. Many thanks also to Mara Ghinelli, Annachiara Tassan and all the Disney staff for their professionalism and imaginative brilliance, which succeeded in rendering our not always intuitive concepts both simple and appealing. Our thanks also go to Cristina Andreani, graphic designer in our Museum, for her help in the creation of bat-t-shirts, and to Alba Scarpellini and Andrea Grigioni, who manage our website. A big “thank you” goes to Marco Riccucci and Mariella Turini (GIRC) for their support and precious bibliographic consultancy. We are also grateful to Mylea L. Bayless and Patricia Brown who reviewed the manuscript, giving us precious suggestions. Last but not least, we thank all the people who took part in our project, supporting our message for the re-evaluation of bats.

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5. From Dracula to Batman: the “BAT BOX: Be a bat’s friend” project as a participatory approach to conserve bats

Paolo Agnelli, Giacomo Maltagliati, Laura Ducci, Stefano Cannicci.

From Dracula to Batman: the “BAT BOX: Be a bat’s friend” project as a participatory approach to conserve bats.

Submitted to Conservation Biology (2012)

5.1 Abstract

Bats (Chiroptera) are an order of mammals that are threatened all over Europe, and generally have a minor appeal to people, which limits the efficiency of conservation programs focused on bats. The “BAT BOX: Be a bat’s friend” project was initiated in 2006, with the aim to distribute bat boxes, which are artificial roosts for bats, and raise awareness in the general public about these animals.

Through public meetings we attempted to change people’s attitudes toward bats and we proposed a tool through which to help us in the conservation of bats. To raise interest, we discussed the ecosystem services that bats perform, in particular the control of insects. From the beginning of the project we distributed about 50000 artificial roosts throughout Italy which were positioned mostly in urban and suburban habitats. People were also trained and recruited as volunteers to collect monthly the monitoring data of their bat boxes. We collected these data yearly and selected those we considered as viable based on sampling effort.

Analysis of the colonization rate with respect to the placement parameters showed that the boxes positioned earlier in time and higher from the ground were preferred over the other boxes. Daily hours of sunlight was also an important feature in the increase of bat box occupation. Initially bat boxes were predominantly colonized during the late summer-early autumn period, while over time bats learned to use artificial roosts during the rest of the year.

Our project spread knowledge about the role, and usefulness, of bats in the ecosystem, and directly involved the public in a conservation program focused on a less appealing animal. In doing so we contributed to change the behavior of people toward bats, making these animals a flagship species for the sustainable control of harmful insects.
KEY WORDS: Artificial roosts, bat boxes, bats, communications, community-based conservation, education, paradigm shift, urban ecology.

5.2 Introduction

Wildlife conservation is a great challenge for conservation biologists, especially for the protection of less appealing organisms. Whilst is relatively easy to encourage the general public to value fascinating animals such as pandas, big cats or birds of prey, and in parallel raise awareness of their important in the ecosystem, this is much more difficult in the case of more unappealing animals. Public interest drives political decisions which often define conservation priorities. For the general public, the prize for the “ugliest animals” goes to invertebrates which often have a low priority in conservation due to their lack of appeal (Beattie & Ehrlich 2010). People are often scared by insects, spiders or jellyfishes and do not appreciate their important role in the ecosystem, often considering them a "problem to solve". Generally, the importance of vertebrate conservation is easier to explain to people and the prize for the "most appealing animals" likely goes to mammals. They are often considered to be fascinating by the general public, perhaps because humans are mammals too, or because some of them are close relatives of the domestic animals with which humans live closely. Bats unfortunately are an exception to this general mammalian-appeal rule, at least in Italy.

Bats are the only active flying mammals. Over about fifty million years they have evolved a number of incredible features which allowed them to live in all habitats worldwide, except Antarctica. About one thousand two hundred species are known in the world and among Mammals the Chiroptera order is second in species richness behind the Rodents. Bats provide ecosystem services such as insect control, pollination and seed dispersal, and they are also good bio-indicators as they generally live in healthy environments (Dietz et al. 2009). Bat populations are decreasing all over the world, and based on a study conducted by the IUCN, most species are considered threatened (Hutson et al. 2001). In the USA, bats are afflicted by an unprecedented disease (the White-Nose Syndrome, WNS) caused by an alien species of fungi, *Geomyces destructans*, which is killing a huge number of cave dwelling bats all over the North American continent (Frick et al. 2010). In Europe, in an attempt to halt the general decline of bats (Bontadina et al. 2008), the Convention on Migratory Species (CMS) established the European Bat Agreement (EUROBATS) which aims to protect all European species through legislation, education, conservation measures and international co-operation (UNEP 1991). However, people generally do not like bats or consider their conservation important (Lunney & Moon 2011). Frequently, people are scared of bats, some because of vampire myths or the threat of rabies, and others simply because of their nocturnal habits. This lack of knowledge is a significant limitation in the conservation of bats.

Changing negative attitudes toward bats through communication and distribution of bat boxes (which are artificial roosts for bats) to the general public was the mission of the "BAT BOX: Be a bat's friend" project which the Natural History Museum of the University of Florence started in 2006 (Agnelli et al. 2011). Through the project and thanks to the help of citizen volunteers we also initiated a participatory study of the colonization rate of the distributed bat boxes.

Our hypothesis was that the colonization of artificial roosts by common crevice dwelling bats is strongly influenced by the parameters of placement. Some of the placement features, such as the aspect, influence the internal temperature that the roost may reach during the day (Brittingham & Williams 2000; Kerth et al. 2001; Laurenço & Palmeirim 2004); other parameters, such as the height from the ground, likely influence the perception of safety by bats (Maltagliati et al. 2009; Agnelli et al. 2011). Some characteristics of the habitat in which the box is placed, for example the proximity to wetlands or green urban areas (Avila-Flores & Fenton 2005), may attract bats because of the proximity to resources (Kunz 1980). Knowledge of the effects of these parameters is important, and can also provide people with some hints on how to install their artificial roosts for a higher chance of colonization. We also studied the effectiveness of our bat boxes in terms of colonization percentages, and evaluated the success of colonization over the duration of the study (Agnelli et al. 2011).

5.3 Materials and methods

5.3.1 The "BAT BOX: Be a bat's friend" project

The project started in 2006 and is still in progress. The bat box model was redesigned according to the size of the Italian species from the construction scheme freely distributed by Bat Conservation International (Tuttle et al. 2005; Agnelli et al. 2011). The production of bat boxes and their distribution was carried out by the Coop, one of the largest Italian distribution companies (Fig. 1b). In order to spread our message, we initiated public meetings to discuss the important role of bats in the ecosystem. In particular, bats contribute to insect density regulation (Ducummon 2001; Kunz et al. 2011), and assist in reducing the problem of mosquitoes in the summer (Rydell et al. 2002; Reiskind & Wund 2009). During the meetings, we employed a multidisciplinary approach to address the topic of mosquitoes based on consultations with chemists, agronomists and experts in pest control. Raising awareness of this aspect of bat biology was one of the major driving forces of our initiative. During the meetings we also explained the objectives of the project and trained volunteers on the placement of the artificial roosts and how to monitor bat colonization.

We also produced an informative brochure containing information about the project and training of volunteers, which was attached to the bat boxes. Since 2010 this brochure has been produced in collaboration with Disney Italy, who created the graphics, enhancing the visual appeal of the document and assisting in shifting public opinion of bats towards a more positive view (Fig. 1c). Disney also created an original character, Kiro, a bat that became a friend to Donald Duck in two published comic booklets. Since the beginning interviews and newspaper articles have been published on the initiative, generally coinciding with the summer increase of mosquitoes. These media sources helped to promote the project, in addition to the traditional communication channels typically used by the Coop. In 2012 we also performed an informative tour in 49 Italian shopping centers where boxes were sold. During this "Chiro-tour", several GIRC (Gruppo Italiano Ricerca

Chirotteri – Italian Chiroptera Research Group) experts met with the public to discuss bats and bat boxes.

Fig. 1. (a) Monitoring form redesigned by Disney Italia; (b) Bat box model distributed since 2011; (c) Cover of the informative brochure redesigned by Disney Italia.

To provide funding support for this project, the Coop also sold merchandising products dedicated to bats and nature. At the end of 2012, the total funds raised during the project were approximately € 180000. These funds are used by the Natural History Museum of the University of Florence to support this and other research and conservation projects on bats, and also financed a three-year scholarship for a PhD project dedicated to the study of these animals.

5.3.2 Bat box monitoring

A monitoring form was distributed, attached to the boxes, to give people a further opportunity to participate in our project. People willingly sent us their data concerning bat box placement and monitoring to participate in the study. Volunteers monitored their boxes by counting guano droppings under the roosts, and the number of individuals inside the boxes. Individual counts were performed by observation from a position below the box with a flashlight; to limit disturbance to bats the roosts could not be opened. The identification of bats is a difficult process, often requiring handling of the animals, and therefore impossible for the general public. Thus, the presence of "bats in boxes" was recorded and no further taxonomic information obtained. Volunteers recorded data on a form, which has been refined over the years based on volunteer's suggestions and the opinions of experts. This form was also available in electronic format and was sent to volunteers upon request by e-mail to the address specifically created for the project (batbox@unifi.it).

We assigned a proper code to each box and these were collated on a specific data base. Monitoring data were sent to us yearly, at the end of November, by mail or fax. The analyses of colonization success were also conducted yearly, and the results were sent via e-mail to the volunteers and published online on the two websites dedicated to the project (www.msn.unifi.it, www.batboxnews.it). These communication tools were also used to inform the general public about news in the field of bat research and to answer their questions about bats. These methods facilitated a connection between the public and the experts, strengthening the relationship between the Museum and the general public.

The monitoring form (Fig. 1a) contained general and contact information of the volunteers who placed the boxes and the main features of the positioning of the roost (date, aspect, daily hours of sunlight during summer, height from the ground and from the floor, proximity to wooded areas and wetlands, installation support and model). Over the years, we refined the requested information to be informative and easy to collect by the volunteers. To evaluate the sampling effort of volunteers, and therefore make a more accurate comparison between bat boxes, we designed an appropriate monitoring table. This consisted of a set of rows for the months of monitoring (April-November), while the columns indicated the decades (which are groups of ten days) of the month. The table we designed was therefore 8x3 with 24 cells, which represented the 24 decades between April and November. Intuitively crossing rows and columns, volunteers filled the table in a certain way: the cell was left empty if they did not perform any checks during the specific decade, while the result of the check was entered into the cell if it was performed during the decade. When selecting the data for analysis, firstly we counted the number of filled cells in every table to evaluate the total number of actual checks for each bat box. Subsequently, we calculated the potential number of checks in the year for every box, based on their date of positioning. We considered viable, and thus we selected as "viable", only those boxes with a number of actual checks which was at least 1/3 of the potential number of checks. Among the viable boxes, we considered all the roosts with at least one successful control as "colonized" (at least one cell with value > 0), either in guano or individual counts.

From 2009 we used the colonization success of boxes as a binary factor to compare the positioning parameters of colonized and not colonized bat boxes with a two-way Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson 2001). Resemblance matrix was based on Gower similarity. To assess the importance of different parameters for the selection of roosts by bats we used a Similarity Percentages test (SIMPER, Anderson et al. 2008) based on a Bray-Curtis similarity. Since 2008, as a result of the two-entry monitoring table, we estimated which months had the highest ratio of successful checks each year. Thus, we could evaluate the month of the year when the bat boxes were most commonly used. To statistically assess any differences in the dynamics of the use of shelters during the years we used a 2x2 contingency table comparing successful and unsuccessful controls in the same month for subsequent years.

5.4 Results

5.4.1 Participation results

One of the aspects of the ecology of bats that most captured the interest of the general public was the large amount of insects that bats consume nightly. We used this aspect to spread correct information regarding the usefulness of these animals in controlling mosquitoes. This encouraged the public to view bats in a positive light and motivated their desire to be involved in their conservation. We therefore distributed bat boxes throughout Italy, thus providing a method for people to contribute actively in bat conservation (Schultz 2011). To date, 1640 volunteers have participated in our study, with 1935 bat boxes currently present in our data base (Table 1).

The number of bat boxes distributed each year has increased significantly thanks to progressive national distribution. The number of volunteers has also increased considerably; for example, between 2009 and 2010 they more than doubled in number. However, the percentage of bat boxes in the database compared to the percentage distributed has decreased significantly over the years since initiation of the project. The percentage of bat boxes monitored followed the general trend of participation in the initiative, remaining around 50% of bat boxes in the database. There was also no significant difference in the percentage of viable bat boxes compared to the monitored boxes, reporting a constant percentage of bat boxes selected (around 80%; Table 1).

An important result, thanks to the participation and collaboration of the public, was the achievement of positively shifting the perception of bats by the general public. This result is difficult to summarize analytically. However, the number of bat boxes sold during the initiative (Table 1) and the success of the sales of other products related to the project for fund raising provides a good index of general interest. This revolutionary and unexpected change of attitude towards bats was also found by many other Italian bat specialists, who thanked us for our initiative which successfully raised awareness for bats in Italy.

Table 1. Bat boxes distributed to citizens and analyzed yearly during the first six-years of our project.

Year	Bb distributed[a] (price)[c]	Bb database[a] (%)[d]	Bb monitored[b] (%)[d]	Bb viable[b] (%)[d]
2007	220 (free)	147 (66.8)	75 (51.0)	-[e]
2008	3220 (25.00 €)	328 (10.2)	192 (58.5)	153 (79.7)
2009	11220 (25.00 €)	537 (4.8)	277 (51.6)	222 (80.1)
2010	27720 (25.00 €)	1213 (4.4)	498 (41.1)	409 (82.1)
2011	41420 (22.00 €)	1640 (4.0)	627 (38.2)	520 (82.9)
2012	50420 (23.90 €)	1935 (3.8)	-[f]	-[f]

[a]Cumulative number of bat boxes (Bb) distributed and collected in our database.
[b]Bat boxes (Bb) monitored yearly and those selected as viable for our analysis.
[c]The price of bat boxes in each year.
[d]The percentages of each value with respect to the value in the previous column.
[e]During 2007 data were not selected.
[f]Data not yet analyzed.

5.4.2 Analysis of bat box placements

The percentage of colonized bat boxes with respect to the viable boxes increased significantly year to year (Spearman's rs: 1.00, $p < 0.05$; Fig. 2a).

The results we obtained in the yearly analysis of the colonization of the bat boxes were consistent over the years. Therefore, we report here only the most recent analysis (2011 monitoring data). The positioning parameters of colonized bat boxes differed significantly compared with those of non-colonized boxes (Pseudo F: 10.07, $p < 0.01$). Based on the SIMPER we found that date (28.84%), height from the ground (19.82%) and daily hours of sunlight during summer (18.93%) explained most (67.59%) of the overall variance.

Considering singularly the most important parameters, we found that the bat boxes positioned for longer had a higher rate of colonization. In particular, the colonization increased linearly with respect to the months since placement of the boxes (R^2: 0.90, Fig. 2b). Similarly, colonization also increased linearly with the increase of the height of positioning from the ground (R^2: 0.93, Fig. 2c). Interpretation of the effect of sunshine received by the roosts during the day on their colonization is less clear. Despite being among the most important parameter, different and unclear trends were observed in all years of monitoring (Fig. 2d).

The months with the highest rate of positive controls were October in 2008, August in 2009 and September in 2010 and 2011. In particular, the comparison of the months in subsequent years showed that between 2008 and 2009, August was the only statistically different month (χ^2: 10.32, $p < 0.01$). The same comparison between 2009 and 2010 revealed that both April (χ^2: 13.97, $p < 0.01$), May (χ^2: 11.07, $p < 0.01$) and June (χ^2: 4.45, $p < 0.05$) were statistically different, whereas no difference was found in the months between 2010 and 2011 (Fig. 3).

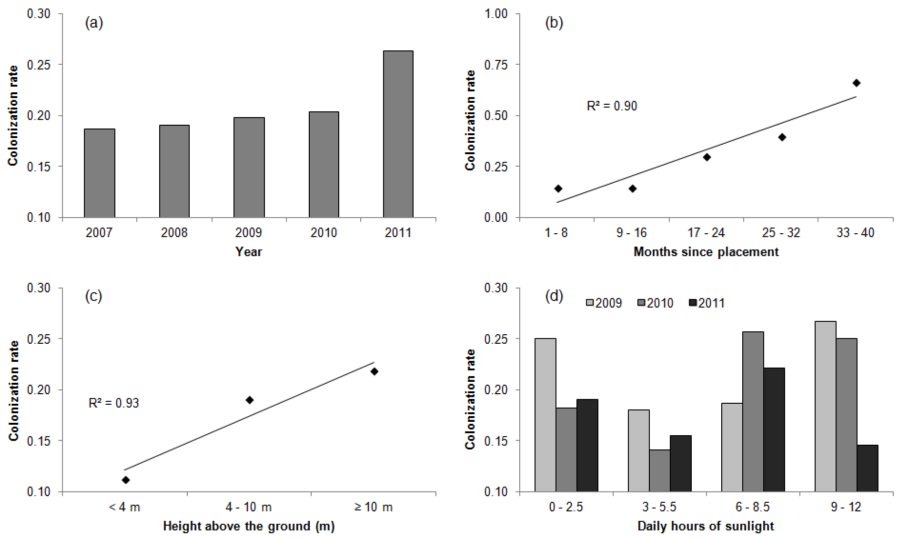

Fig. 2. (a) Yearly percentage of colonized bat boxes with respect to those selected as viable; (b) Colonization rate of bat boxes clustered by months due to their different months of placement, R^2 value is also given; (c) Colonization rate of bat boxes clustered by height above the ground, R^2 value is also given; (d) Colonization rate of bat boxes clustered by hours of sunlight received during the day, reported by years.

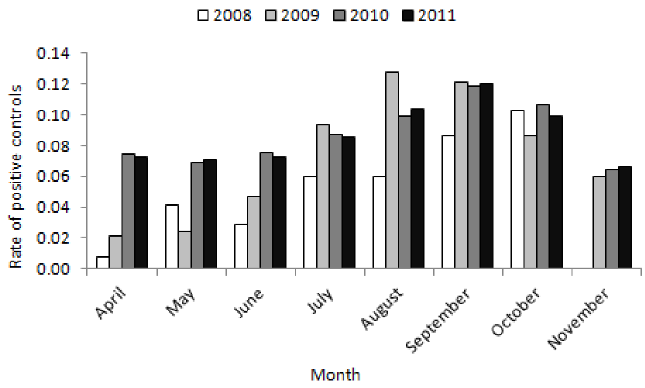

Fig. 3. Monthly rate of positive controls (counts with either number of guano droppings or individuals > 0) calculated in the various years.

5.5 Discussion

5.5.1 People participation and project

Changing positively the behavior of the general public (Schultz 2011) and their views of bats was certainly the biggest challenge of this project. Therefore, the success in the distribution of bat boxes throughout Italy and the participation of volunteers in our study was our greatest achievement. In fact, many of the volunteers were even adverse to bats before joining our project.

To achieve this result it was necessary to communicate with people, using language without scientific technical terms. Our message was then delivered using appropriate communication tools such as illustrated brochures, web or newspaper articles and television interviews. To improve the effectiveness of our message (Stern 2000) and increase participation in the project, we found a particular aspect of the ecology of bats for which, even from a practical point of view, it is particularly important to protect these animals.

This aspect was the ecosystem service that bats play in regulating the density of nocturnal flying insects, such as mosquitoes (Reiskind & Wund 2009; Kunz et al. 2011). Every year, during summer, people complain about the stress caused by these insects and many resources are spent to manage this "problem" (Pimentel 2009). Generally, this leads to a huge release of harmful chemical compounds which, in fact, fail to solve the problem, due to increasing pesticide resistance in pests (Roberts & Andre 1994).

Thanks to a major communication effort we were able to raise awareness of the benefits of bats in the biological control of these insects. Through meetings, brochures, articles and interviews we also discussed more generally the correct integrated approach to managing mosquitoes during the summer. Firstly, we defined as a priority a reduction in the use of chemicals, which are more hazardous for us and predators of insects rather than for the insects themselves (Pimentel 2009). We then discussed alternative and sustainable techniques oriented towards control of larvae and adults. We suggested some easy methods for everyone to make their own small contribution to managing the problem of mosquito larvae. Furthermore, to manage adult mosquitoes, we suggested an easy method to assist in the conservation of their natural predators, such as bats, and introduced the concept of placing artificial roosts (Agnelli et al. 2011). We thus made the bats a "flagship species" (Veríssimo et al. 2011) for the integrated biological control of mosquitoes.

One of the driving forces for the project was to be able to participate closely with the general public who, in fact, were the principal actors in our conservation program.

Using volunteers in environmental monitoring is not a new initiative across various taxonomic groups (Schmeller et al. 2009). One of the main benefits of this method is the economic aspect, which allows the collection of a large amount of data on different spatial and time scales with a very low budget. On the other hand, appropriate training of volunteers by experts is necessary (Macdonalds et al. 1998; Toms et al. 1999). Newman et al. (2003) compared the effectiveness of a participatory monitoring project on small and large mammals with one performed by experts in the UK. The study involved a small area where volunteers, divided into small teams, performed the tasks usually performed by experts. Researchers trained volunteers continuously and, although in many tasks the volunteers had a lower efficiency compared to specialists, they collected good quality data displaying improvement over time. However, some tasks resulted unsuitable to be carried out by people with no experience.

A more participatory approach at a smaller scale was conducted by Goffredo et al. (2004) to study the distribution of seahorses in Italian seas. In this study, volunteers were non-professional scuba divers who were trained, through thematic days, how to recognize the target species and to conduct observations. The researcher's task was limited to training people during single events, and to maintain contact with them during the entire duration of the study. This "single-event" approach for the training was allowed due to the simplicity of the requested task.

Volunteers are not very frequently used in the study of bats but this approach is not, however, an innovation. UK national bat monitoring is regularly carried out to evaluate the consistency of the bat populations, also through the collaboration of volunteers. These volunteers are people with an interest in the study of bats, or who share their home with a nursery colony and therefore help to protect them (Walsh et al. 2001). However, our monitoring program conducted entirely by volunteers with no previous experience and with a conservation purpose on such a small scale is unprecedented in the study of bats. The frequency of monitoring in our study, and thus the amount of data collected, was very high compared to similar studies performed entirely by experts.

A study by Whitaker et al. (2006) which involved the placement of 3204 bat boxes during eight years as a mitigation tool near the Indianapolis International Airport is probably the study with the largest number of bat boxes monitored. They were generally checked once a year, with a maximum of about 4000 annual checks in the 1994-1997 period. In our project, 10258 checks were carried out in 2011 alone by our staff of volunteers on a smaller number of roosts (627) with a frequency varying from a maximum of three checks per month to a minimum of one a year. The necessity of such a high monitoring frequency to properly evaluate the colonization of these shelters was confirmed by the great variability in the use of our artificial roosts by bats throughout the year, especially during the first years after placement. Data collected sometimes had inconsistencies which were usually resolved through direct interviews. We rejected any data which remained fragmented or unclear.

5.5.2 Analysis of bat box placement

The success of colonization of bat boxes in similar projects is quite variable (White 2004; Ciechanowski 2005; Flaquer et al. 2006; Lesinski et al. 2006), and our results (Fig. 2a) are the first for urban and suburban areas (Agnelli et al. 2011). This particular habitat is frequented by some of the most common species of bat, which use crevices in human buildings as roosts. These species are generally considered to be at a lower risk of extinction (Hutson et al. 2001); however they are an important component of urban biodiversity.

The positioning parameters of the bat boxes significantly influenced the success of colonization (White 2004). The parameters that were found to be relevant, and their effects on colonization, were confirmed during all of the years of our study. The time since box positioning was the factor that had the most significant effect on colonization of the roosts. This factor likely also affected the increase of colonization over the years (Fig. 2b). Bats, in fact, actively search for new roosts during their nightly activity (Dietz et al. 2009), and they may need some time to locate a suitable roost. However, colonization is not only linked to the location of a potential roost. Bats choose their shelters depending on the season of year and their physiological requirements (Lausen & Barclay 2002). A roost found in a particular period of the year may only be used later in time, when environmental conditions are suitable for its occupation. In some cases, people reported bat boxes which were used initially as a night-roost and only later as a daily roost. This usually happens for the colonization of roosts by isolated individuals. Nursery colonies of females that have used the bat boxes generally return year after year, and thus significantly increase the number of individuals using that particular box. This increase may be due to the birth of female pups which return as adults, but it could also be explained by an increasing degree of safety that bats attribute to a roost which attracts other individuals. Perceived safety of the roosts can also be considered as the reason that bats preferred boxes higher from the ground. The presence of terrestrial predators (including humans) able to reach their shelters present a sufficient threat to these animals, given their high vulnerability during the daily resting period. The height of the roost from the ground is probably an effective barrier against many terrestrial predators which, unlike the aerial predators, cannot reach such shelters without some footholds (Agnelli & Guaita 2009).

Although there is no clear explanation, the daily hours of sunlight which the shelters receive appears to be important in roost choice. This is probably related to how this parameter directly affects the internal temperature of the bat boxes. The internal microclimate is particularly important in the choice of shelters by bats because of their daily torpor, performed during the day to save energy (Grinevitch et al. 1995). In our study, the preference for shelters with different exposure to sun varied over the years, and no general trend can be summarized (Fig. 2c). This high variability may be caused by the ability of bats to switch roosts to suit their needs in the various seasons. Doing so, they maximize energy savings related to daytime torpor (Lausen & Barclay 2002). For instance, there are significant differences in this behavior between males and pregnant females during the reproductive season (spring-summer). Males are solitary and require colder shaded shelters, while pregnant females aggregate in sun exposed roosts, forming nursery colonies to warm each other (Grinevitch et al. 1995). The fact that bat boxes were colonized during all months of the monitoring period, by both isolated individuals and colonies, may have resulted in this unclear preference for sunshine.

Nevertheless, the dynamics in the use of artificial shelters throughout the year highlighted some differences in the occupation through seasons. In the various years, the months in which the bat boxes were most used were those between the reproductive and mating season (summer-autumn). During this period, the recently weaned juveniles disperse from the roost in which they are born and therefore require new shelters to spend the day. Similarly, females leave the roosts where they gave birth and begin to visit those actively defended by territorial males, which are often polygynic (Dietz et al. 2009). Therefore this is a period of colonization of new roosts for bats, and may explain the large occupation of our bat boxes. From year to year, however, we registered some differences in the yearly dynamics. In 2008, we recorded an increasing trend in the use of bat boxes from early spring until the October peak. In 2009, this trend was slightly reduced and the peak was anticipated in August, which had a significantly higher occupation than August of the previous year. In 2010 and 2011, this trend had almost disappeared and there was a significant increase in the spring months compared to 2009, despite the peaks in September. It appears that over the years the bat boxes were gradually occupied earlier in the year and now our artificial roosts are frequented similarly during all months of the monitoring period. This is probably related to the loyalty that bats show to permanent shelters (Lewis 1995), especially to those that have proven to be safe and thermally suitable.

Our project transformed a super-villain that the public believed to be capable of harm, into a super-hero assisting them in their daily struggle against harmful insects. This was achieved through an accurate and motivational message, which was divulged using proper multiple communication tools.

We also trained people to assist their new hero, directly promoting the presence of bats through the placement of proper roosts and spreading knowledge to others. Our project allowed volunteers to change their behavior and to be directly involved in the conservation process. Some people also contributed to improve the effectiveness of the project by participating in the study of the placing parameters. The data we collected were not complex, but of good quality and time- and cost-effective. The study improved our knowledge regarding the colonization of bat boxes in urban and suburban habitats, and our results helped people to achieve more successful placements. In conclusion, our participatory approach to collect information to drive our adaptive management of the project was an effective way to involve people directly in the conservation of bats, which are now “more appealing mammals”.

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6. The role of aspect and color in the colonization of urban bat boxes: a two-year study

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The role of aspect and color in the colonization of urban bat boxes: a two-year study.
Under review in Journal for Nature Conservation (2012)

6.1 Abstract

Bat boxes are an increasingly used tool for the conservation and study of bats; thus it is important to understand which factors influence the colonization rate of these artificial roosts. Some studies investigating colonization of boxes by nursery colonies have verified that the internal temperature that the roost reaches strongly influences the success of colonization. These female aggregations are generally used as a model to evaluate the success of bat boxes, and in particular their importance for conservation purposes. Little is known about the preferences of males and non reproductive females. Similarly, there are few studies that consider the use of these artificial roosts in the urban environment, even though it is commonly frequented by some species of bats.

This study assesses how the exposure and color of bat boxes, factors that directly affect the internal temperature of the roost, influence the roost choice by bats living in the urban environment. The bat boxes were used throughout the year, including winter. The roost choice was mainly affected by the interaction between orientation and season; therefore it was not possible to delineate a general preferred orientation, as this varies with season.

Color was a significant factor affecting the colonization of boxes by males and non reproductive females which preferred lighter roosts in autumn and winter. During five years of continuous monitoring no nursery colonies were observed to colonize bat boxes. Therefore, our results can only be interpreted within the context of conservation of males and non reproductive females. However, this study provides important information to assist in the improvement of urban bat box positioning in future conservation programs.
KEY WORDS: Artificial roosts, bats, roost choice, urban habitat

6.2 Introduction

For many years European bat populations have been increasingly in decline (Hutson et al. 2001; Bontadina et al. 2008). This crisis is so alarming that recently the Convention on Migratory Species (CMS) established the European Bat Agreement (EUROBATS), which promotes measures to protect European bats. This population decline can be largely attributed to an increase in human environmental impact, such as air and water pollution, habitat loss and fragmentation, and loss of suitable bat roosts both under and over ground (Hutson et al. 2001). However, human presence can favour some species of bat, and these species constitute some of the most valuable components of the degraded urban ecosystems. These bats, which are tolerant to human presence, survive by taking refuge inside human artefacts and using urban green spaces as foraging areas, where they assist in regulating insect density (Avila-Flores &

Fenton 2005). Thus, they provide an important ecosystem service to citizens (Kunz et al. 2011), and it is important to encourage the presence of these valuable insect predators, even in degraded habitats, such as urban areas, in an attempt to counter the negative human impact that prevents or limits their presence (i.e. loss of roosts in building; Brittingham & Williams 2000).

One of the most utilized tools in both the study and conservation of bats are bat boxes, which are artificial roosts, and through their monitoring provide a means to gather information about the bat fauna in a given area (Stebbings & Walsh 1991). The colonization rate of bat boxes is variable (White 2004; Ciechanowski 2005; Flaquer et al. 2006; Lesinski et al. 2006), largely in response to certain factors often related to positioning parameters and technical characteristics of the roosts (Stebbings & Walsh 1991). One of the most important parameters affecting the colonization of bat boxes is the internal temperature of the shelter (Kerth et al. 2001; Laurenço & Palmeirim 2004; Bartonička & Řehák 2007). The placement aspect, and thus the daily exposure to sun, and the color of a bat box directly influence the internal temperature that this roosts will attain during the day (Brittingham & Williams 2000; Kerth et al. 2001; Laurenço & Palmeirim 2004). These factors, therefore, appear to be important for colonization, since bats require roosts with different microclimatic conditions throughout the year, and the internal temperature plays a key role in their choice (Kerth et al. 2001; Lausen & Barclay 2002; Rancourt et al. 2005; Flaquer et al. 2006). Individuals may also face different ecological needs within a season; for example, females require warmer roosts during the reproductive period to speed up embryo development (Grinevitch et al. 1995; Wilde et al. 1995), while non-pregnant females and males use cooler roosts to facilitate daytime torpor (Grinevitch et al. 1995). Darker boxes can reach higher internal temperatures, particularly if south-facing, and this should encourage colonization by nursery colonies in this period (Kerth et al. 2001; Laurenço & Palmeirim 2004); conversely, light colored roosts, and those which are shaded and cooler, should be favored by isolated individuals (both males and non reproductive females) for torpor facilitation (Hamilton & Barclay 1994).

To study how these afore-mentioned factors could affect artificial roost colonization in urban areas, we utilized the bat box model created by the Natural History Museum of the University of Florence (Agnelli et al. 2011), initiating a study which specifically aimed to evaluate if the placement and color of bat boxes affects their colonization. We conducted a monitoring program for two years on artificial roosts of two colors (light and dark) positioned facing the four main orientations on a building. Our hypothesis was that bat boxes with a higher internal temperature (dark and south-oriented) would be chosen during the period of gestation and weaning (spring and summer) by the nursery colonies. By contrast, in the same period we expected that cooler roosts (light and north-oriented) would be chosen by males and non reproductive females. We also hypothesized a general preference for the cooler boxes, thus being favourable for daily torpor, in autumn and winter when females that gave birth in spring also need to enter into torpor in the daytime.

6.3 Materials and methods

On June 15 2007 we placed eight bat boxes on four sides of the astronomical tower of the Natural History Museum of Florence. This small astronomical tower, located on the top of Torrigiani Palace, is one of the highest towers above the city of Florence (about 40 m above the road surface) and is located next to the Boboli Gardens, a large urban green area. This part of the Museum is never frequented by visitors and thus remains undisturbed throughout the year. This building was built at the end of the 18th century, and the astronomical tower has an octagonal shape. For the placement of bat boxes, we selected the four facades that are the best approximates of the four cardinal directions. We placed two bat boxes on each of the four facades, one dark and one light, at about three meters above the tread surface that surrounds the tower.

The bat boxes used were single-chambers made of 1.0 cm thick marine plywood planks. The boxes were 76.0 cm long, 39.0 cm wide and 4.5 cm thick. The bottom entry fissure and the internal crevice had a depth of 2.5 cm, while the access ramp was 10.0 cm long. There was also a horizontal vent in the lower portion of the boxes. This bat box model was redesigned according to the size of the Italian species from the construction scheme freely distributed by the American association Bat Conservation International (Tuttle et al. 2005). A model of such size ensures an appropriate vertical stratification of temperature, and the lower vent creates a zone with a lower internal temperature to optimize behavioral thermoregulation during hotter days (Brittingham & Williams 2000). All boxes were properly treated for outdoor positioning with water-based products, and the dark boxes were painted with a water-based dark brown paint.

The first colonization occurred in mid April 2009, almost two years after the placement of boxes, with two individuals counted in the dark west-oriented bat box. We waited one year to allow bats to locate the different roosts and from April 2010 the bat boxes were checked three times a month, approximately once every 10 days, throughout the year (with the exception of December 2011, when only two checks were performed). The monitoring consisted of a count of excrements below the box and direct observation inside the box, through a quick visual check with a flashlight from below the roost. This method was chosen to minimize the disturbance to the bats inside the roost (White 2004; Lesiński et al. 2006); however, it did not allow us to identify bats to species level. During each control session we counted and removed any droppings and we counted individuals inside the bat box. We adopted both counting techniques as they are complementary. In winter, a count of droppings is not an accurate method to detect the presence of individuals due to the low metabolic level of hibernating bats, and the consequent low production of guano. Conversely, in other seasons the presence of droppings below a roost can represent night-roosting and/or past occupancy of the box. Due to the small number of bats that occupied the boxes, we never performed a visual count of their emergence. Data collection was completed in late March 2012.

Data were stored in a data base. Variation in individual counts and dropping counts recorded between April 2010 and March 2012 were analyzed statistically with a three-way PERMANOVA design (Anderson 2001), with orientation, season and color of the artificial roosts as orthogonal and fixed factors. When applicable, pairwise post-hoc tests were also applied.

6.4 Results

All eight bat boxes were colonized at least once during the monitoring period (Table 1). Total number of droppings counted in winter was extremely low (24) compared to other seasons (spring: 499; summer: 483; autumn: 404), while total number of individuals counted was quite similar across seasons (winter: 63; spring: 29; summer: 25; autumn: 65). Based on the visual checks, we were able to assign all of the bats we counted to the genus *Pipistrellus* Kaup, 1829 or *Hypsugo* Kolenati, 1856, the most common crevice dwelling bats in Italy.

Table 1. Successful checks (number of droppings or individuals > 0) out of a total of 71 counts for each bat box in the March 2010 - April 2012 period (D: dark; L: light)

Orientation	Color	Succ. checks	%
N	D	25	35.2
	L	23	32.4
E	D	18	25.4
	L	23	32.4
S	D	13	18.3
	L	24	33.8
W	D	39	54.9
	L	47	66.2

The most visited bat box was the lighter west-orientated, while the least visited was the darker south-orientated. The highest numbers of droppings (estimate of 35) were counted in September 2010 (lighter west- and darker east-orientated), October 2010 (darker south-orientated) and April 2011 (lighter south-orientated). The highest number of individuals (5) was counted in December 2010 in the lighter west-orientated. The average numbers of bats counted in all boxes per check during each season were: winter 3.71; spring 1.61; summer 1.39; autumn 3.61. We never found pups, or any evidence of nursery colonies, inside the roosts.

Orientation and season significantly affected the pattern of artificial roost use, both individually and in their interaction. Color significantly affected the use of bat boxes, both alone and in interaction with season. The interaction of all three parameters was also statistically significant (Table 2).

Table 2. PERMANOVA test on differences in color (co), orientation (or) and season (se) in number of individual and dropping counts during the year. All factors are treated as fixed and orthogonal. The degrees of freedom, df; Mean Squares, MS, value of the Pseudo-F statistic and its probability level, p, are shown.

Source	df	MS	Pseudo-F	p
color – co	1	3.49	3.94	< 0.05
orientation – or	3	5.27	5.96	< 0.01
season – se	3	16.86	19.07	< 0.01
co x or	3	1.77	2.01	> 0.05
co x se	3	2.34	2.64	< 0.05
or x se	9	5.03	5.69	< 0.01
co x or x se	9	2.66	3.01	< 0.01
Res	536			
Total	567			

Pairwise tests between orientation and season showed that in winter west-oriented boxes were significantly preferred over other orientations (N: t 4.13, $p < 0.01$; E: t 3.86, $p < 0.01$; S: t 2.32, $p < 0.01$); in summer the south-orientated boxes were avoided (N: t 5.90 $p < 0.01$; W: t 3.62 $p < 0.01$; E: t 3.54 $p < 0.01$) and the north-orientated boxes were preferred also over the east-orientated boxes (t 2.24 $p < 0.05$); in spring the west- (t 2.60, $p < 0.01$) and east-oriented boxes (t 2.23, $p < 0.05$) were preferred over the north-orientated boxes; in autumn west-orientated boxes were preferred over the north- (t 2.93, $p < 0.01$) and east-orientated boxes (t 4.08, $p < 0.01$) and the south-orientated boxes were preferred over the east-orientated boxes (t 2.05, $p < 0.05$; Fig. 1).

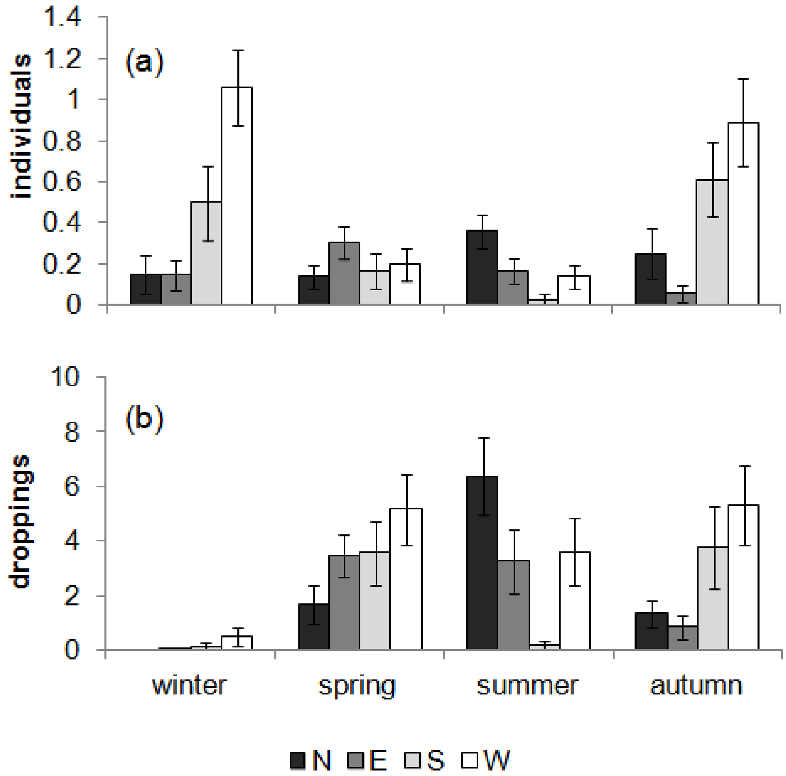

Fig. 1. Mean number (± SE) of individuals (a) and droppings (b), clustered by aspect, counted inside and under boxes in various seasons.

Between color and season the pairwise tests were statistically significant in autumn (t 2.19, $p < 0.05$) and winter (t 3.11, $p < 0.01$), when light roosts were preferred (Fig. 2).

Pairwise tests performed to test the interaction between all three factors showed that color was statistically significant for the west-oriented boxes in autumn (t 2.85, $p < 0.01$) and winter (t 2.07, $p < 0.05$), for the south-orientated boxes in winter (t 2.35, $p < 0.05$), for the east-orientated boxes in summer (t 2.08, $p < 0.05$), and for the north-oriented boxes in summer (t 2.30, $p < 0.05$). In all cases, except north-orientated boxes in summer, bats preferred lighter roosts.

6.5 Discussion

All of the bat boxes were visited at least once by bats; however, the first colonization did not occur until almost two years after placement. This implies that conservation projects may need several years to accurately evaluate colonization success.

The count of excrements alone as an occupation index leads to an underestimation of roost colonization in winter months, thus integration with visual checks of individuals is necessary. Visual inspection also allows the identification of bats in artificial roosts at genus level. The *Pipistrellus* and *Hypsugo* genera are the most common among crevice dwelling bats in Italy (Lanza & Agnelli 2002), and are an important constituent of the biodiversity, albeit-low, in urban areas.

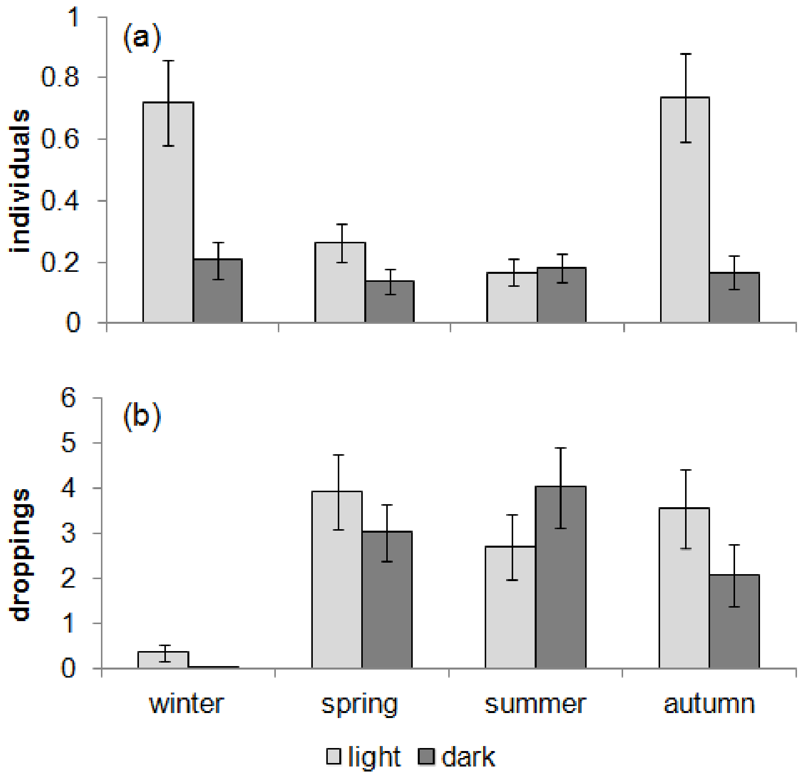

Fig. 2. Mean number (± SE) of individuals (a) and droppings (b), clustered by color, counted inside and under boxes in various seasons.

None of the boxes were colonized by nursery colonies, even though the roosts were positioned to differentiate the internal microclimate as much as possible; previous studies have shown that these roosts can be colonized by groups of pregnant females (Brittingham & Williams 2000; Laurenço & Palmeirim 2004). Some of these studies, however, refer to boxes placed very close to roosts already used by pregnant females, which were forced to abandon their original roost due to eradication by homeowners (Brittingham & Williams 2000) and, consequently, likely relocated to bat boxes. White (2004) found a low colonization rate by nursery colonies compared to isolated individuals in various environments. Whereas Laurenço and Palmeirim (2004) reported a high colonization rate by nursery colonies in bat boxes placed near wetlands. It is likely that this discrepancy is due to local environmental characteristics, such as the proximity to foraging areas or wetlands. These are very important factors in roost choice by nursery colonies. In fact, these colonies use warmer roosts during the day, resulting in a great loss of water through evaporation (Kunz 1980), and they also need to forage close to the roost to minimize the energetic cost of returning to pups at night for suckling (Davis et al. 1968; Williams & Brittingham 1997). In our study, the proximity to Boboli Garden, which is a good foraging area for bats with numerous locations for water consumption, was probably not sufficient for the selection of our bat boxes by maternity colonies. Further studies which compare roosts used and unused by nurseries will clarify the main factors affecting the colonization of bat boxes by maternity colonies in urban areas. However, in the present study we considered how aspect and color may influence the roost choice of males and non pregnant females.

In accordance with our hypothesis, the orientation of bat boxes, and thus the daily sun exposure, plays an important role in roost choice. However, it was not possible to determine which exposure is generally favoured by bats, since preferences vary between seasons and so they may often switch roost (Lausen & Barclay 2002). Nevertheless, there was a general preference for west-oriented bat boxes in at least two seasons of the year, and for north-orientated boxes in summer. This preference is probably related to the dynamics of the daytime torpor. During torpor bats lower their metabolism and temperature during the day, emerging from torpor at sunset when they must leave their roosts to feed (Dietz et al. 2009). The most energetically expensive phase of this strategy is the rewarming which bats perform to emerge from torpor (Prothero & Jurgens 1986). West-oriented roosts may support a passive rewarming, receiving direct sunlight later in the day at the time when bats should wake up (Hamilton & Barclay 1994). In fact, these roosts are shaded and cooler when bats need to maintain low metabolic rates, while they are sun exposed and warmer when bats need to wake up for their nocturnal foraging activity. This energy saving strategy seems to be apparent, especially during winter months, in our study. Some species of the *Pipistrellus* genus, in fact, do not migrate to hibernacula in winter, instead they stay in summer roosts where they lower their activity and frequently they still arouse on warmer nights (Avery 1985). The absence of hibernation in such species in the urban landscape is also likely favoured by the microclimate, which is warmer in winter than in near habitats (urban heat island effect, Manley 1958; Parris & Hazell 2005) and by a general change in climate. The urban buffered seasonality may favour a higher winter arousal frequency in bats that arouse more often on warmer nights when their prey availability is higher (Avery 1985; Hope & Jones 2012). We also found that the average number of individuals per check was higher during autumn and winter, which is generally the time we consider individuals to abandon this type of roost, and thus monitoring is often not carried out. Bat box monitoring programs in urban and suburban environments that do not include cooler months may thus lead to a general underestimation of the actual occupation of artificial roosts. In our study south-oriented roosts were avoided during summer, probably because of excessive internal temperatures due to constant sunlight, which prevent non-pregnant females and males from entering into torpor. In fact, during summer the north-oriented roosts were generally preferred.

Color is an important factor for the colonization of artificial roosts by males and non pregnant females (White 2004) in autumn and winter only, when dark roosts were avoided. This is not the case for nursery colonies, as shown by Laurenço and Palmeirim (2004) in *Pipistrellus pygmaues* (Leach 1825) and Kerth et al. (2001) in *Myotis bechsteinii* (Kuhl 1817). They demonstrated that darker roosts favour the presence of large groups of pregnant females, which require high temperatures to accelerate embryo development. In our study it was not possible to confirm this hypothesis due to the absence of nursery colonies in our boxes. However, females that form nursery colonies stay together in spring and summer only, and at the end of this period they either live individually or in small groups. Therefore it is likely that even those females choose cooler roosts after the pups weaning.

Considering color, aspect and season together it appears that light roosts are generally preferred at all aspects, at least in certain seasons. The only exception was darker north-orientated which was preferred over lighter north-orientated in summer. Further studies to compare the temperature inside roosts of various colors and aspects may help to clarify this preference. Again it seems that the torpor behavior displayed by bats during the day plays an important role in roost choice by males and non pregnant females.

The present study confirms the effective use of bat boxes by bats in urban environments (Agnelli et al. 2011). However, the absence of colonization of these single-chamber artificial roosts by nursery colonies, despite the theoretical ideal positioning, questions the effectiveness of such artificial roosts to offer refuge to these larger aggregations in strictly urban environments. We have also shown that west-oriented boxes allow a higher colonization rate in most of the year, due to the occupancy of non reproductive females and males. Light-colored roosts were preferred by males and non reproductive female, whereas dark roosts are preferred by nursery colonies (Kerth et al. 2001; Laurenço & Palmeirim 2004). Bats frequently switch roost to fulfil their changing requirements throughout the year, and thus there is no general preference in color or aspect of positioning.

Artificial roosts have proven to be a critical tool for the conservation of bats in urban landscapes, and the present data bridge the gap in our knowledge concerning their colonization by non-reproductive specimens throughout the year. In the context of the development of bat conservation programs in urban habitats, our results strongly suggest that the deployment of multiple-roost clusters of both dark and light bat boxes facing as many aspects as possible must be considered for the successful colonization of these artificial roosts.

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7. CONCLUSIONS

This research was focused on the study of some important aspects of the behavior and ecology of human-tolerant bats. These species share the ability to adapt their habits to the presence of humans, tolerating a variable degree of stress which is caused by the human intervention on the environment. This plasticity sometimes make these species more common or simply easier to come across with respect to other ones which are strictly related to more pristine habitats. The presence of human-tolerant species in urban and suburban areas may lead to a misjudgement, considering them less important or less interesting because less vulnerable. I think instead that a better understanding of their ecology may be very useful in the conservation of all the species of bats, at least in Italy, where most of the landscape is highly influenced by the human intervention.

I studied the choice of roosts both in crevice dwelling bats (Chapter 4, 5, 6), which may also frequent the urban landscape to forage, and in those species which need large volumes to roost in buildings (Chapter 2) that usually frequent only the less disturbed suburban areas.

Both groups needed multiple roosts throughout the year and their choice was mainly influenced by the internal microclimate and by the risk of predation. Moreover the switch of roosts was assessed to be a frequent behaviour even in these species, both in response of biological and climatic seasonality and to avoid predation. Saving energy for termoregulation and selecting safer roosts were the main aspects that influence positively the choice of shelters by human-tolerant bats. Traditionally, the conservation of nursery colonies through the maintenance of the roosts, mainly used during the reproductive and hibernating season, was considered an effective method to protect bat populations. My study highlighted the necessity of the conservation of multiple roosts, apart the ones which are primarily used for reproduction and for hibernation, that, however, remain the main focuses for the bats conservation.

Nursery colonies which aggregate in variable numbers during the spring-summer period are considered a main concern for the conservation of bats, mainly because the low reproductive rate of these animals. In fact, in temperate zones, bats usually grow one or two pups each year. Better understanding the nightly emergence behavior of such aggregations, which is a moment of high vulnerability for bats, may thus help the conservation of bat populations (Chapter 2, 3). In *Rhinolophus ferrumequinum* the emergence resulted to be anticipated in dimmer conditions and in larger colonies, especially in presence of pups, when females have an higher trophic necessity due to lactation. Juveniles tend to be less prone to risk, emerging later at night and using the roost as a flight training camp. The light pollution, which drastically alter the perception of dark at evening in bats, is therefore a major threat for such animals, especially for those species which live in urban and suburban areas where artificial illumination is high. Even the landscape mosaic which is present near the roost appear to influence the emergence behavior, probably changing the diet and thus the hunting habits of this species.

I also studied the efficiency of artificial roosts for bats in urban and suburban areas (Chapter 4, 5). Bat boxes were used in fact both to study the preference of crevice dwelling bats and to give those species a good alternative to the "traditional" roosts in buildings. This is the first study about the colonization of such artificial roosts in urban areas at a small scale. My results will help the management of future similar projects, suggesting bat conservationists about the production, placement and monitoring of bat boxes. The "BAT BOX: Be a bat's friend" project is also an unprecedented program to raise people interest and knowledge about bats.

Through the involvement of people in its project, the Natural History Museum of the University of Florence succeeded to change the bad attitude toward bats in most of the general public that it reached with its message. It made people aware about their stakeholder role in the conservation of bats and allowed them to participate actively in its project to do something for their wellness, albeit indirectly. This was possible through the spread of a correct message which captured the people interest: bats eat a lot of mosquitoes and therefore may help us in controlling these insects. This particular aspect of the biology of bats led to a paradigm shift in their perception and made them a flagship species for the biological control of insects. However, using properly the mass media to spread a correct message was not an easy task. Journalists often alter information to make them more appealing while people tend to listen just what they prefer. Communication is a powerful tool for conservation, but a lot of experience is needed to properly use this kind of resource to spread correct information.

Raising people awareness about a topic also allowed to gather economic resources by private companies which willingly found such projects to promote their active role in the conservation of the environment. Green topics are often sustained by companies which increasingly invest in a sustainable development.

Conservation biology is a complex science which need various expertise to be exploited. The study of conservation ecology (populations, species, ecosystems) is the basis on which to decide a proper conservation strategy and the study of behavior is also important for the animal conservation. Lack of knowledge in specialists, decision makers and people, constitutes one of the biggest limit to conservation. Proper techniques to communicate science to the various stakeholders are therefore needed. Gathering economic resources in the private sector is also important, especially in this period of dramatic crisis of public Research investment in Italy. In conclusion, through a multi-disciplinary approach which involved ecology, ethology, communication and economics this study was able to contribute significantly to the conservation of a group of species of bats which were shifted in perception from negative to positive in just a few years.

Acknowledgements

I am really grateful to Paolo, Laura and Cosimo who supported, trained and made me enjoy this work with their passion and expertise. I thank also Stefano, who left his crabs for a while to take a walk on the bats world.

Many thanks to the whole staff of the Natural History Museum of Florence (Annalisa included) with whom I celebrated too many birthdays and thanks to my GIRC colleagues, especially Martina, Marco, Maria Tiziana and Mauro, and to Saulo and Francesco for the cover pictures of this thesis.

Thanks also to the many people and the Coop staff who participated in the "BAT BOX: Be a bat's friend" project, especially Maura, Antonio, Roberta, Bernardo and Silvia, and thanks to the staff of the Natural Park of Migliarino, San Rossore, Massaciuccoli, especially Antonio and Olga.

Finally thanks to my family, to pre- and post-graduate colleagues at "La Specola", especially Alberto and Giulia, to the Grizo, which is a part of me, to the rest of my friends and to Alessandra for "the love" (Lonigro 2012). You all supported me every single day, thank you.