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## **Lesser Horseshoe Bat *Rhinolophus hipposideros* (André, 1797)**

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### **Taxonomy and systematics**

The lesser horseshoe bat (Fig. 1) was first recognized as a distinctive, smaller form of the greater horseshoe bat (*R. ferrumequinum*) during the 1700s (e.g., de Buffon 1760; Pennant 1771; Schreber 1775). Although Buffon recognized the bigger and smaller forms, he kept them as one species as he was not certain that they truly represented two species rather than age classes or intraspecific variation. Later, it was formally named and described as a species in its own right (*Noctilio hipposideros*) by André in 1797. Bechstein (1799) or Borkhausen (1797) are regularly credited for describing the species. Refer to Benda and Mlíkovský (2022) for a detailed overview of the nomenclature and validity of taxonomic names and publication dates for this species. The type locality for this species is often given as France, but the justification for this is not clear. Being unaware of the then recently published literature, Hermann (1804) also wrote a species description, as did Montagu (1808), in a very detailed and lively account.



**Figure 1** Lesser horseshoe bat (*Rhinolophus hipposideros*). ©Wolfgang Forstmeier.

## Subspecies

Multiple subspecies have been described for the lesser horseshoe bat starting with Andersen (Andersen, 1907, 1905a, 1905b) who described three forms in Europe based primarily on average forearm length: the larger nominate form occurring to the north, a slightly smaller form (*minimus* Heuglin, 1861) to the south (Mediterranean – later limited to Eritrea and Crete), and a further smaller form (*minutus* Montagu, 1808 occurring in England, Wales and Ireland). Andersen readily acknowledged that the *minutus* form was likely an extreme western offshoot of the continental source populations which developed a smaller body size (Andersen, 1905a). He further correctly predicted that intermediate forms and a transition zone would be found between the two forms occurring in continental Europe (Andersen, 1907). The decision to name subspecies based on such small differences in average measures with unknown taxonomic relevance reflects a different time. Miller (1912) did not find that there was sufficient material to reach a satisfactory conclusion on the imperfectly differentiated forms. Further subspecies were described by Andersen : *midas* Andersen, 1905a (Iran), *majori* Andersen, 1918 (Corsica) and *escalerae* Andersen, 1918 (Morocco). These have been upheld by some authors, with additional justification based on skull morphology and potentially karyotype (discussed in Benda et al., 2006). However, strong justification for recognition of subspecies is rarely given.

It is known that lesser horseshoe bats exhibit great geographical size variation, and that well defined geographic clines exist even across small areas of the distribution (e.g. within France) probably related to climatic conditions (Saint-Girons and Caubère, 1966). See also Salinas-Ramos et al. (2021). Saint-Giron and Caubère further analysed the reported measurements for the then described subspecies across Europe and concluded it was impossible to distinguish two subspecies. There was no distributional limit of clearly different forms, with intermediate forms being common and the variation in size existing on a geographic cline running southwest (smaller) to northeast (larger), with populations living in mountainous areas (Pyrenees, Alps) also being larger. A phylogeographic study on this species (see section on Phylogeography) included individuals from localities relevant to the described subspecies mentioned above: sites across continental Europe, Britain, Ireland, Iran, Corsica, Crete and Morocco. There was no genetic support for the recognition of subspecies. As is the case for other horseshoe bat species with known geographic variation (e.g. *R. mehelyi* this volume, Puechmaille 2020), it would seem prudent to regard the lesser horseshoe bat as a monotypic species until there is convincing evidence of the contrary.

The earliest systematic work on horseshoe bats was presented by Knud Andersen (Andersen, 1905a). Based on morphological examination, he arranged the specimens available to him into six ‘types’ or species groups, noting that *R. hipposideros* had no closely related ally, aside from *R. midas* (which is now considered either as a synonym or subspecies of *R. hipposideros*; see section on subspecies). This distinctive position has remained unchanged up to the present despite large numbers of newly described *Rhinolophus* species and several focused studies on the family.

This distinctive systematic position of the lesser horseshoe bat as a monotypic group within Rhinolophidae is well supported by genetic distances based on allozyme data (Qumsiyeh et al., 1988), morphology (Bogdanowicz, 1992; Bogdanowicz and Owen, 1992), and nuclear

and mitochondrial genetic sequence data (Demos et al., 2019; Dool et al., 2016a; Foley et al., 2015; Guillén-Servent et al., 2003; Stoffberg et al., 2010).

The broad phylogenetic structure within Rhinolophidae consists of an Afro-Palaeartic clade sister to a species-rich Asian clade, with the addition of one or more basal clades containing a few Asian taxa. The basal nodes of the phylogeny have not been recovered consistently or with high support to date. The position of *R. hipposideros*, which is often basal, is variable and never highly supported. Its position is thus considered unresolved at present. Without exception however, it is consistently recovered as a divergent branch within the family with no extant close relatives, just as Andersen had originally surmised. The lineage of *R. hipposideros* diverged from its closest relative approximately 16 million years ago (Dool et al., 2016a).

### **Paleontology**

Rhinolophidae diverged from Hipposideridae ~42 MYA during the Eocene (Foley et al., 2015). Horseshoe bat fossils are known from the late Eocene of Europe, the Miocene of Africa, and the Pleistocene of Asia (refs. in Bogdanowicz and Owen, 1992). During the Miocene the family radiated, with the earliest currently known fossils attributable to *R. hipposideros* appearing during the Pliocene, Spain (Agustí et al., 2011). More recent fossil records are known from across Europe, including some Mediterranean islands, and neighbouring regions during the Pleistocene. For example, Spain: Pliocene and late Pleistocene (Agustí et al., 2011; Galán et al., 2016); Ibiza: upper Pleistocene and Holocene (Alcover, 2003). France: middle and late Pleistocene (Jullien, 1972; Sevilla, 1990; Sevilla and Chaline, 2011). Italy: early, middle and late Pleistocene (Salari and Kotsakis, 2011; Tata and Kotsakis, 2005); Sardinia: upper Pleistocene (Kotsakis, 1987). Malta: middle Pleistocene and Holocene (Storch, 1974). Austria: middle Pleistocene, Pleistocene/Holocene and Holocene (Rabeder, 1972; Sapper, 1977; Nagel and Rabeder, 1992; Döppes and Rabeder 1997; Spitzenberger and Bauer, 2001). Poland: later Pleistocene and Holocene (Ochman, 2003; Woloszyn, 1987). Hungary: upper Pleistocene (Topál, 1981). England: Holocene (Yalden, 1986). Palestine: mid to late Pleistocene (Boutié, 1979). Morocco: Holocene (López-García et al., 2013).

### **Current Distribution**

The lesser horseshoe bat is widely distributed in southern Europe, being found in all countries bordering the Mediterranean Sea and on all the major Mediterranean islands (Fig. 2). It occurs throughout Turkey and the Balkan peninsula north into southern Poland and Ukraine. Following a severe range contraction in the 1960s it is largely absent from Germany, although there are small populations restricted to the east (in Saxony, Saxony-Anhalt and Thuringia) and along the country's southern border. It occurs in Slovakia, Czechia, Austria, and Switzerland. Although notably absent from areas of Brittany, it is widespread in France and occurs in areas of Belgium. The populations in Britain and Ireland both have a south-west distribution.



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**Figure 2.** Distribution of *Rhinolophus hipposideros* in Europe, based on the IUCN Red List of Threatened Species, Version 2021-2 and second Report by the United Kingdom under Article 17, JNCC (2008). Map template: ©Copyright Getty Images/iStockphoto

### Description

The lesser horseshoe bat is the smallest of the European Rhinolophidae, and weighs 4 – 9 g with a wingspan of 225 - 250 mm. In common with all members of this family, it has a horseshoe-shaped nose-leaf around its nostrils that comprises a semi-circular disk of flesh at the base, which covers the upper lip. A flat pointed fleshy protrusion, the lancet, emerges above the nostrils and forms the top of the nose-leaf. The nostrils themselves are separated by forward pointing folds of skin, the sella and the connecting process. Although the species can be generally distinguished from the other European Rhinolophids on its size alone, the sella of the lesser horseshoe bat is relatively longer and more pointed in profile than the other species and the connecting process is relatively short and rounded. The lower lip has a single groove. The ears, which are highly mobile when the bat is active, are roughly triangular in shape and 10 – 12 mm in length. This species lacks a tragus but has a broad antitragus.

The pelage in adult animals is buff brown in colour with the ventral fur being slightly paler than the dorsal. Prior to the first moult, at roughly one year of age, the fur is grey (Gaisler, 1966). The pelvic girdle in Rhinolophids is underdeveloped, and the lesser horseshoe bat is no exception, the hind legs are particularly spindly and weak with small feet. This makes quadrupedal locomotion almost impossible, although this species can slowly drag itself backwards over horizontal surfaces and it will use its hind legs to climb into vertically opening crevices in rock. At rest lesser horseshoe bats hang free from the roosting surface by their feet. They have broad short wings, the wing membranes are dark glossy brown in colour. When active in the roost the wings are held semi-wrapped around the body, in torpor

or hibernation, they totally enclose the body of the bat. The uropatagium completely encloses the tail, which at rest is folded back parallel to the spine.

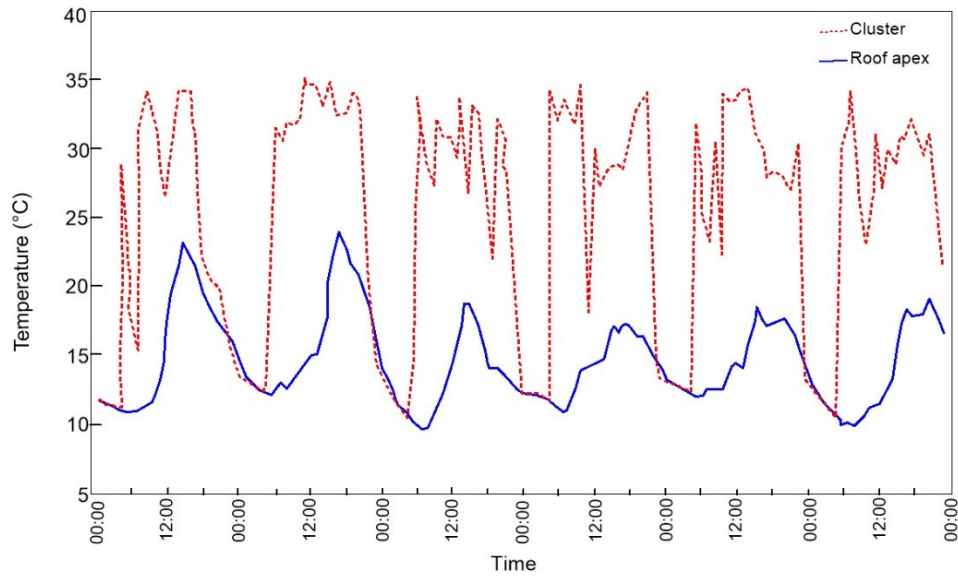
The forearm of lesser horseshoe bats generally ranges from 35 to 39 mm. Females being slightly, but significantly, larger than males (Dietz et. al 2006,). There are size variations across the range of this species (see Subspecies), with individuals at higher latitudes and altitudes being slightly larger than their counterparts in more southern areas of their distribution and at lower altitudes (Salinas-Ramos et. al 2021).

The skull of the lesser horseshoe bat is delicate with a bulbous swelling behind the large opening into the nasal cavity. The condylobasal length is 13.5 – 15.2 mm. The dentition formulae are: upper count 1.1.2.3, lower count 2.1.3.3. The length of the upper (maxillary) teeth row is 5.2 – 5.6 mm, the length of the lower (mandibular) row is 5.2 -5.8 mm.

### Physiology

Lesser horseshoe bats regulate their energetic demands by following the typical annual cycle of other temperate bat species, going into deep torpor during depths of winter and actively thermoregulating a high body temperature during the spring, summer and early autumn months, whilst retaining the ability to enter a daily torpor during periods of inclement weather or low prey availability (Altringham, 2011). The use of daily torpor to balance energetic needs is not a problem for males. However, the continued maintenance of a high body temperature is critical for females during pregnancy and lactation (Racey et. al 1987). Entering torpor at these periods in their reproductive cycle will slow the development of the foetus, resulting in a longer gestation period and later births, with the subsequent problems of a lack of time to build up fat reserves prior to hibernation for both mother and young. In addition, entering torpor during the lactation period reduces milk production with the resulting threat of starvation for the pup. A key component of energetic costs is thermogenesis, although this is not a problem when bats are flying, as muscle contraction generates body heat, when roosting the energetic demands on the bat will be related to the ambient temperature of their refuge ( $T_{\text{roost}}$ ). This in turn is related to the external ambient temperature ( $T_a$ ), which is affected by factors such as latitude and altitude. Ideally, the bats should be selecting  $T_{\text{roost}}$  that is in their thermoneutral zone, minimising energy expenditure on thermogenesis or behaviours to reduce their body temperature (Speakman and Thomas, 2003).  $T_a$  varies across the species' range, with those colonies at higher latitudes and altitudes, being subject to the greatest challenge in generating body heat, whilst those at lower latitudes may be selecting roosts that remain cooler. An indication of the thermoneutral zone for lesser horseshoe bats may be inferred through the temperature found within clustering bats in maternity colonies. This behaviour is observed during the cooler parts of the day in roosts in buildings and almost continually in cave roosts, where the colony huddles tightly together and shares body heat; reducing the cost of thermogenesis compared to roosting separately (Gilbert, 2010). The mean temperature within clusters of lesser horseshoe bats is 30.9°C (Schofield, 1996), this was 14.6°C warmer than the temperature at a control point 0.5m away (Fig. 3).





**Figure 3.** The temperature recorded hourly over a six day period at the cluster point and a control 0.5m away

Even with the temperature buffering effect of the roost building and the heat sink provided by roofing materials, mean roost temperatures of around 30°C are rarely encountered at higher latitudes, and so clustering behaviour must supplement the solar gain. The larger colonies sizes observed in buildings at higher latitudes and altitudes, or in cave roosts in central Europe, may reflect this offsetting of energy demands. Where it is available the colonies will utilise supplementary heat sources by roosting close to chimneys in roof voids, or heating boilers and hot water pipes in lower parts of a building.

## Genetics

### Chromosomes

Bats rarely exhibit intraspecific chromosomal variation and in the temperate zone, variation within genera is also limited (Zima 2004). Within Rhinolophidae, Asian clade taxa generally have  $2n=62$  (with some notable exceptions) whilst those in the Afro-Palaeartic clade have  $2n=58$  (Sotero-Caio et al., 2017; Volleth et al., 2017; Zima et al., 1992). The lesser horseshoe bat is somewhat of an exception with three chromosomal races described. These broadly occur in a geographic cline:  $2n=58$  in the east of the species distribution,  $2n=56$  in eastern Europe and  $2n=54$  in western Europe (reviewed in Volleth et al., 2013; Zima et al., 1992). Approximately 50 individuals have been karyotyped to date, with the  $2n=58$  variant known from Turkey, Syria, Iran, and Jordan (Arslan and Zima, 2014; Benda and Horáček, 1998; Qumsiyeh et al., 1988, 1986; Zima, 2004), the  $2n=56$  variant from Bulgaria, Czech Republic, Greece, Italy and Slovakia (Belcheva et al., 1990; Capanna, 1968; Hanak et al., 2001; Volleth et al., 2013; Zima, 1982) and the  $2n=54$  variant from Ireland, Spain, Germany, and Switzerland (Kacprzyk et al., 2016; Matthey and Bovey, 1948; Puerma et al., 2008; Volleth et al., 2013). Records not conforming to this clinal pattern include Turkey ( $2n=54$ , Karataş et al., 2006), and Azerbaijan ( $2n=56$ , Fattajev, 1978 as cited by Puerma et al. 2008, Zima et al. 1992). However, as noted by (Arslan and Zima, 2014; Volleth et al., 2013) the Turkish record is not equivalent to the typical  $2n=54$  karyotype in terms of number and sizes of metacentric

pairs; the record from Azerbaijan is from an unpublished thesis which is not easily accessible. A recent study by (Kacprzyk et al., 2016) demonstrated that fibroblast cell cultures suitable for karyotype analyses could be grown from 3 mm membrane biopsies which substantially opens the possibility for increased geographic sampling of this species. Coupled with the latest banding techniques and FISH, this non-lethal protocol could enable clarification of outstanding issues in this otherwise karyotypically well-studied species.

The  $2n=56$  karyotype consists of 23 acrocentric chromosomal pairs, and 3 metacentric which differ in size (one large, two medium-sized and one tiny). The X chromosome is medium-sized submetacentric and the Y is small and submetacentric (revealed by C-banding; using conventional staining it appears dot-like) (Volleth et al., 2013). In the  $2n=54$  karyotype, two acrocentric chromosomal pairs (6 and 23 of the  $2n=56$  variant) have fused to form an additional large submetacentric chromosome pair (Volleth et al., 2013). The  $2n=58$  karyotype lacks large metacentric autosomal pairs and instead consists of two medium-sized metacentric pairs and 26 acrocentric pairs (Arslan and Zima, 2014; Qumsiyeh et al., 1986).

These karyotypic races are thought to have evolved by simple Robertsonian fusions/fissions, which perhaps took place during colonisation (Volleth et al., 2013). Fixation of chromosomal changes can occur rapidly in isolated or colonising populations (King, 1993). At present two contact zones can be assumed (between  $2n=54$  and  $2n=56$  and between  $2n=56$  and  $2n=58$ ). The F1 generation would be simple heterozygotes for a single Robertsonian fusion. Simple heterozygosity does not typically cause a problem during meiosis and infertility is unlikely (Baker and Bickham, 1986; Bulatova et al., 2011); the different karyotypic forms can be maintained as chromosomal polymorphisms. The phylogeographic study (Dool et al., 2013 discussed above) demonstrated high levels of gene-flow and little differentiation within the West clade ( $2n=54$  and  $2n=56$  variants) and recent gene-flow between the West and East clade ( $2n=58$ ). The study included individuals which had previously been karyotyped: Rhip\_357 and Rhip\_350 are NMP 48712 and NMP 48710 respectively, both  $2n=56$  (Hanak et al., 2001); Rhip\_352 and Rhip\_353 are NMP 48056 and NMP 48054, both  $2n=58$  (Benda et al., 2006; Benda and Horáček, 1998). Genetic divergence (cytb) between presumed karyotypic races was previously noted to be within the normal range for intra-specific variation (Guillén-Servent et al., 2003).

### Phylogeography

The species' phylogeographic history was reconstructed using a multi-locus dataset from approximately 400 individuals sampled across the species distribution (Dool et al., 2013). The markers used included a nuclear intron on the X chromosome (Bgn, 543 bp), mitochondrial DNA (cytb-tRNA-control region; 1630 bp) and eight variable nuclear microsatellite loci, chosen to provide distinct temporal snapshots of the species' demographic past.

The intron dataset revealed that the source of early colonisations was from the east (Turkey and further east) where genetic diversity was highest, which was similarly inferred for *R. ferrumequinum* (Flanders et al., 2009). In contrast, all populations sampled in Europe and North Africa shared a single high frequency haplotype, or one closely related to it (1-2 mutations). Interestingly, the origin and colonisation routes taken by lesser horseshoe bats were already suggested by Andersen based on the simple examination of specimens (Andersen, 1905a). The mtDNA data showed that *R. hipposideros* used multiple glacial



refugia throughout the Mediterranean. This included at least three refugia in the east (Turkey, Cyprus, and a clade with individuals from Lebanon, Syria, Turkey, and Israel), with further refugia in southern Italy/Malta, Morocco, Tunisia, Southern Iberia, Northern Iberia, the Balkans, and Crete. The source refugia for the West clade is far from clear and it is possible that more recent or short-lived refugia existed in Europe across the northern Mediterranean. Mitochondrial diversity was highest in these inferred glacial refugia and lowest in Britain and Ireland at the limit of the colonisation front. Despite strong geographic structure in the mtDNA, the microsatellite dataset demonstrated recent gene-flow between all populations, with the sole exception of Iberia versus the rest of Europe. Indeed, there was strong support for restricted gene-flow occurring across the Pyrenees. In accordance with these results, the same pattern was found for the tick species *Ixodes vespertilionis*, whose main host is *R. hipposideros*: limited mtDNA structure within Europe, but high differentiation between Spain and France (Hornok et al., 2015). The grey long-eared bat (*Plecotus austriacus*) was also found to have low levels of genetic exchange across the Pyrenees (Razgour et al., 2013), though this pattern does not appear to be particularly common in the bat species which have been studied to date.

The phylogeographic study demonstrated the importance of sampling the full species range as far as possible, as the source population and most of the refugia were not found in mainland Europe. With improved protocols and reduced costs of non-invasive genetics (discussed below), there are now great opportunities to conduct fine-scale sampling to improve the currently poor sampling of the genetically diverse eastern populations and to determine detailed colonisation routes across north Africa and the Mediterranean sea.

### Population genetics

Numerous studies have generated mtDNA sequences for *R. hipposideros* as part of studies with broader geographic or taxonomic goals (e.g. Ibanez et al., 2006). However, there are also several studies which have focused specifically on within-species diversity and structure to address a range of questions relevant to this species. The earliest of these studies was facilitated by the development of microsatellite loci specifically for *R. hipposideros* (Puechmaille et al., 2005) based on non-invasive sampling. The feasibility of applying this protocol to large-scale non-invasive studies was demonstrated by (Puechmaille et al., 2007) who achieved a >90% success rate for mtDNA sequencing (338 bp Cytb, 586 samples) and >95% for microsatellite amplification (12,592 PCRs). Some of these same DNA extracts were later used as part of the phylogeographic study discussed above (mtDNA >800 bp Dool et al., 2013). The optimised microsatellite protocol was further used to accurately assess population size using a non-invasive capture-mark-recapture (CMR) estimation method (Puechmaille and Petit, 2007).

Reliable population censuses are absolutely critical for effective conservation management. Based on a single sampling event (i.e. collection of droppings from three maternity roosts), highly accurate estimates of population size were achieved. Accuracy was validated by 2-7 consistent visual counts for each site. This study proved that without capturing or disturbing bats, guano can be collected and used to generate high quality DNA and to accurately estimate population sizes quickly and relatively cheaply. The microsatellites were also used to assess genetic diversity and connectivity, census size and to assign genotypes (from ~ 900 droppings) to unique individuals in 20 maternity colonies in Franche-Comté, eastern France (Afonso et al., 2016). The droppings from this study were also used to test for the presence of

metals, pesticides and a protozoan parasite (*Eimeria hessei*) as indicators of colony health. 449 individuals were sampled and the faecal prevalence of *E. hessei* was found to correlate with increasing concentrations of Cd and Zn in droppings, which were interpreted as an indication of chronic exposure to metals rather than acute contamination (Afonso et al., 2016). This study demonstrated the potential the applications of non-invasive genetics to aid in the characterisation of pollutant-pathogen interactions and the consequent impacts on bat and colony health.

The same microsatellites were then used to characterise the effects of habitat fragmentation in the population with the lowest genetic diversity of any sampled so far (Dool et al., 2013). Lesser horseshoe bats are known to be a woodland associated sedentary species (e.g. Bontadina et al. 2002; Reiter, 2004a; Reiter et al., 2013), however, due to extensive deforestation, Ireland has very little remaining broadleaf woodland cover. Individuals were sampled from 37 maternity colonies covering the species distribution in Ireland, which is restricted to the west of the country (Dool et al., 2016b). Using mtDNA, microsatellites, echolocation calls and habitat suitability modelling, population differentiation was strongly supported across datasets. Differentiation into two populations was caused by reduced gene-flow due to a gap in the species distribution. Estimates of effective population size were very low and there was evidence for historic population declines in 31 of the 37 colonies (by 3-37%). There was an indication of further genetic differentiation of the four most northern colonies which also exhibited unusually high, and seemingly maladaptive, peak frequencies considering atmospheric attenuation.

The drivers of genetic diversity were examined by studying 42 colonies across the leading edge of the European distribution using microsatellites and a modelling approach (Lehnen et al. 2021). The results showed that historic bottlenecks impacted genetic diversity but that contemporary connectivity best explained current genetic diversity at a 65 km radius around the site. Furthermore, gaps greater than 60-80 km are predicted to result in loss of connectivity between colonies (Lehnen et al. 2021).

With the costs of high-throughput techniques becoming ever lower, methods of genotyping, including those based on non-invasive sampling, have become increasingly feasible and appealing. This has catalysed a second wave of population genetics studies on the lesser horseshoe bat based on non-invasive sampling. A new protocol was developed in which each step was high-throughput and could be largely automated: plate extractions of droppings (96 samples at a time), amplification a sex-linked locus in addition to the available species specific microsatellites in a single reaction, and genotyping and automated analyses performed using a custom script (Zarzoso-Lacoste et al., 2017).

The addition of the bioinformatic pipeline ensures rapid and unbiased genotyping even when datasets are large. The protocol was validated using droppings collected before and after parturition from 19 maternity colonies in Picardy, northern France. DNA extracts were rapidly genotyped and sexed, resulting in the identification of 1,337 unique individuals from 3,544 extractions. The population size was estimated based on CMR; thereafter parentage was estimated, and sex-related behaviours were investigated (e.g. probability of roost occupancy based on sex or reproductive status in the case of females). The total costs of the study amounted to €7.25 per sample for consumables and two months of lab work and analysis, demonstrating that non-invasive genetic sampling can now be readily expanded to

large datasets and in an affordable manner in terms of time and finances. This protocol has already been used to validate a novel approach using Approximate Bayesian Computation to accurately and rapidly infer the sex ratio of populations from acoustic data alone (Lehnen et al., 2018); which is possible due to the sex-based differences in lesser horseshoe bat echolocation calls (Jones et al., 1992). The high throughput protocol was also used to develop a new approach based on non-invasive CMR data to estimate adult survival, fecundity and juvenile survival - key parameters of population growth (Jan et al., 2019a). Droppings were collected from 35 maternity colonies in two regions to cover an expanding population and a stable population. The reproduction rate was found to be higher in the expanding population and was hypothesised to be due to higher resource acquisition enabled by favourable environmental conditions in the novel habitat (discussed in Population Ecology).

### Hybridization

No existing data suggest hybridization between *R. hipposideros* and any other species.

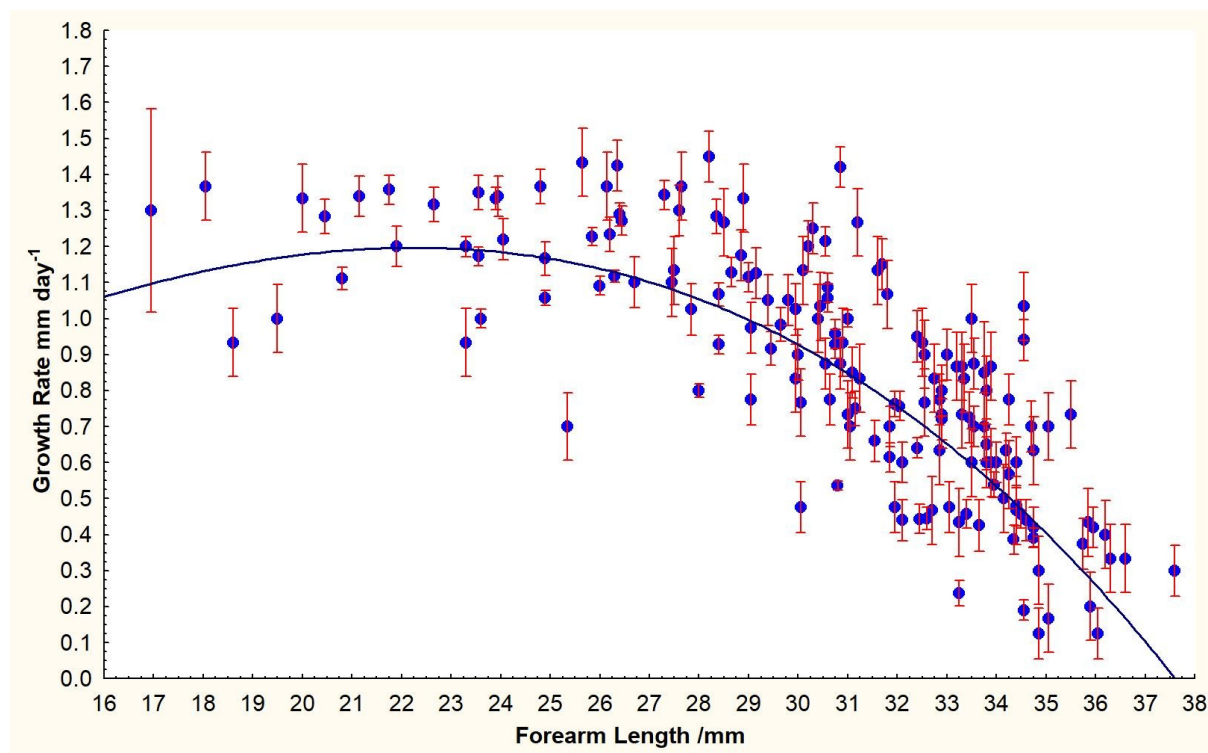
### Life History

Lesser horseshoe bats follow the annual cycle common to most European bats with the winter months spent in hibernation followed by an active period in the spring, summer and early autumn when breeding occurs. The conventional method for determine the age span in bats is by ringing juveniles and subsequently recapturing them. However, lesser horseshoe bats do not react well to rings, which can cause high levels of mortality, consequently data on longevity using this method is limited and may be skewed towards ages that are less than their true life expectancy. This is reflected in the unsustainably low mean longevity of 2.3 years determined by Sluiter (1960) following the mass ringing of lesser horseshoe bats in the caves of South Limburg in the 1950s. Subsequently ringing of the species was severely curtailed in the 1960s and is still banned in some European countries (Dietz et. al. 2006). However, where ringing has continued there are a few exceptional recaptures of individuals after 14 years 6 months (Herreid, 1960), 21 years (Harmata, 1982) and 21 years 10 months (Presetnik and Trilar, 2013).

The majority of mating in lesser horseshoe bats takes place in the autumn and early winter prior to the bats entering hibernation, with a smaller proportion occurring in early spring when they emerge from their winter quarters (Gaisler, 1965). Following copulation, a plug is formed in the vagina, secreted by the male, and this prevents further insemination. The sperm is stored in the uterus until ovulation, which occurs in April, at this point the vaginal plug is ejected. Maternity colonies form in late April or early May, depending on local climate and weather conditions. Gestation in lesser horseshoe bats is long, around 75 days (Gaisler, 1966) but this is also subject to weather conditions. Consequently, the timing of births is strongly influenced by spring temperatures (Schofield, 1996, Reiter, 2004a). Births in a colony are spread out over a three-to-four-week period with the first births in central and western Europe typically occurring from late June to early July (Reiter, 2004a), it is likely that this is earlier in the warmer climates of southern Europe. Although there have been histological studies exceptionally showing twin fetuses in the uterus, there are no records of births of twins in lesser horseshoe bats and Gaisler (1966) speculates that the second foetus may be reabsorbed. This makes sense as the pup is large at 34% of the lean body mass of females (Schofield, 1996). The mean forearm length of the pups at birth reflects the clinal size variation in the species across Europe with those in the Britain having mean forearm length of 16.7mm and a mean mass of 1.85g compared to 17.5mm and 2.0g in Austria (Schofield, 1996, Reiter,

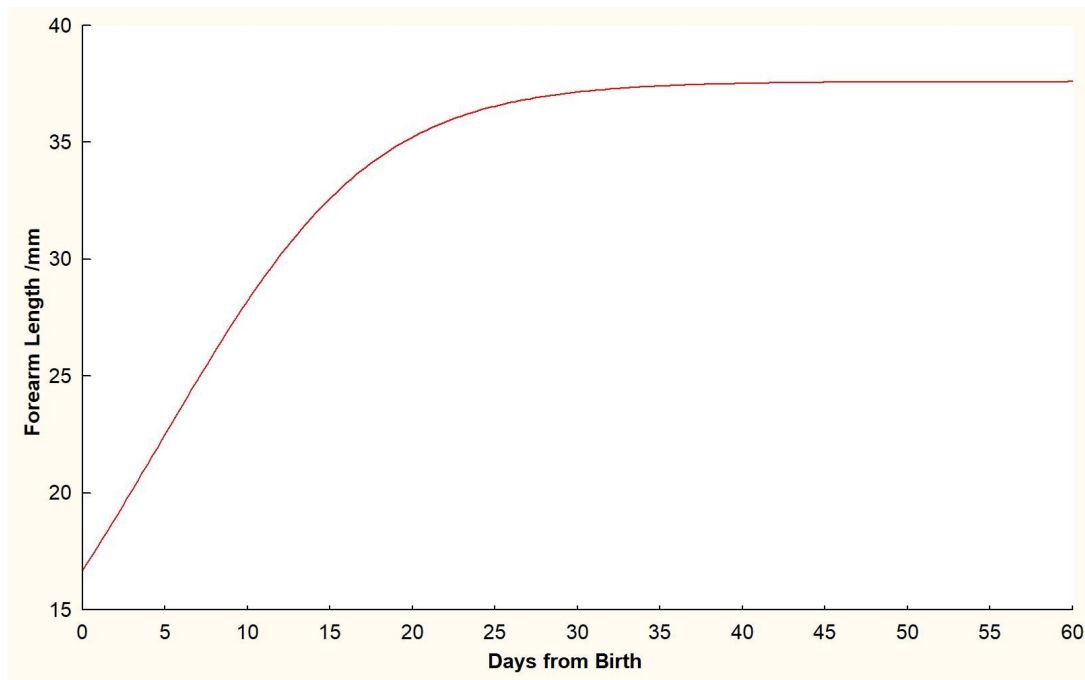
2004a), no detectable differences are recorded between the sexes in the forearm length or mass at birth. Females develop false nipples in the pelvic region after birth and these are used by pups to grip with their teeth and grasp around mother's neck with feet so they can be carried around the roost and when the mother is foraging in first week or so following birth until the pups get too large to carry. These false nipples remain visible following first birth and can be used as an indication that female is parous.

As with the length of gestation and timing of births, climatic factors can influence the growth rate of pups post-partum. Reiter (2004) showed that periods of inclement weather reduced the growth in forearm length, an effect that was more pronounced in pups born later in the colony. Overall, the growth rate of lesser horseshoe bat pups is high with a Bertalanffy-Richards growth rate curve parameter  $k$  of 0.075 for the forearm length and 0.142 for mass (Schofield, 1996; Fig. 4). Few other bat species have a growth rate higher than lesser horseshoe bats (Kunz and Stern, 1995; Kunz and Hood, 2000).



**Figure 4.** A scatterplot of the growth rate points for mean forearm length. Closed circles are the growth rates ( $dL/dt$ ) at the mean forearm length between recaptures of bats during post-natal growth. The solid line is the fitted curve of the Bertalanffy-Richards growth rate equation.

At around 10 days and with a forearm length of about 28mm the pups start excising their wings, grasping the mother around the head and neck with their feet and rapidly flapping their wings. Pups with forearms of 34mm are able to fly short distances but sustained flight only occurs when the forearm reaches 36mm at 23 days. The pups grow rapidly, approaching the forearm length of adults at around 40 days but epiphyses are not fully fused until around 70 days (Schofield, 1996; Fig. 5).



**Figure 5.** The increase in forearm length in lesser horseshoe bats during post-natal growth. The line represents the growth curve from the Bertalanffy-Richards equation.

Male lesser horseshoe bats do not become sexually mature until they are one year old, at which time the size of their testes and the microscopic structure of the sex organs cannot be used to separate them from adults from previous cohorts (Gaisler 1966). Spermatogenesis begins in the spring and is at its peak in July and August before ending as the bats enter hibernation, however, the tubules of the epididymis remain filled with spermatozoa over the winter and into the following spring (Gaisler and Titlbach, 1964). Sexual maturity in female lesser horseshoe bats can occur as early as 4 months with a small number of bats giving birth when they are one year old, however, for the majority this occurs when they are 2 and in small numbers a year later at three years old (Gaisler, 1966). Pregnancy does not occur every year and may be related to body condition emerging from hibernation.

## Habitat and Diet

### Roosts

Like all bat species in Europe, the lesser horseshoe bat uses roosts during the day and for hibernating. Historically this species is believed to have roosted in caves throughout the year (Issel 1950; Gaisler 1963a; Kovalyova 1997), which is still the case, in the southern parts of its global range (Schober and Grimmberger 1998; Schofield 2008; Dietz et al. 2016). However, a few records indicate that large hollow trees may also have been used as roosts by this species (Gaisler 1963b).

In the northern parts of its distribution *R. hipposideros* predominantly uses man-made structures. Maternity roosts are typically found in attics of churches, castles and other larger historical buildings (Gaisler 1963a; Reiter et al. 2004; Schofield 2008; Dietz et al. 2016). However, maternity roosts of this species can also be found in smaller attics of private

buildings and even in hollow concrete bridges (Gaisler 1963a; Reiter et al. 2004). Moreover, lesser horseshoe bats take advantage of heat sources within buildings and thus, are able to use underground compartments for successfully raising their young. Such heat sources include boilers, heating pipes or the waste heat from turbines in power stations (Issel 1950; Gaisler 1963a and 1963b; Reiter et al. 2004).

Interestingly, for most countries in the Mediterranean region, there are few records of maternity roosts in caves (Benda et al. 2008; Nagy and Postawa 2011; Presetnik et al. 2014). However, it is unknown whether this is because maternity roosts of lesser horseshoe bats in caves are overlooked, because they use small caves which are seldom visited by bat experts (Presetnik and Knapič 2014) or because caves are usually visited during winter.

Only a very few maternity colonies in underground sites are known from the temperate zone. A maternity roost in Thuringia is located in a cave in the karst area of Kyffhäuser (IFT 2002; Biedermann et al. 2006) and a similar roost is reported from Slovenia (Presetnik and Kumar 2012). In some cases the findings of a group of adult and young lesser horseshoe bats in underground sites (Baar et al. 1986) could in fact be a satellite roosts from a corresponding maternity roost (Reiter et al. 2004).

For the small maternity roost in a cave in Slovenia Presetnik and Knapič (2014) recorded a mean temperature of 18.9 °C (STD = 3.5, Min = 11.9 and Max = 27.4°C). In comparison to external temperatures the average cave temperature was not different from the general climate. However, minimum temperatures in the cave were significantly warmer, and both cave maximum temperatures and daily temperature oscillations were significantly reduced compared to ambient conditions (Presetnik and Knapič 2014).

Lesser horseshoe bats are highly selective in the overground maternity roosts they use (Schofield 2008). However, the selected roost types are dependent on availability and hence, vary in different landscapes. In Britain, *R. hipposideros* uses roosts in buildings from the 19th century, with stone walls and slate roofs, and direct entrances through open doors, windows or holes in walls (Schofield 2008). Maternity roosts of this species in Austria were characterised by complex structured attics and open entrances (Reiter et al. 2004). Roosts of lesser horseshoe bats in Austria frequently have high light levels and darkness seems to be of less importance (Reiter et al. 2004). Another important factor for the colonisation of buildings is the lack or a low level of human disturbance (Schofield 2008; Reiter et al. 2004). This is a factor common for most bat species in Europe, but as lesser horseshoe bats often roost in very open and exposed situations it is of particular concern (Schofield 2008).

For maternity colonies of *R. hipposideros* on a small Mediterranean island Winter et al. (2020) found that, colony size and proportion of reproductive females were higher and parturition started earlier in larger, warmer, and drier nurseries. However, roosts are not selected based on their quality alone. Factors like the distance to foraging areas, the connectivity to foraging areas and the proportion of suitable foraging areas around the roost are important for the selection of maternity roosts by this species (Schofield 2008; Reiter 2004b, Winter et al. 2020). More recently, a new factor appears to be important for the suitability of roosts for lesser horseshoe bats: the external illumination of buildings. Since this species is very sensitive to high light levels, external illuminations of buildings may render them unsuitable or negatively influence emergence times and the use of flight routes.



During summer *R. hipposideros* uses a number of roosts with different functions. Maternity roosts play a central role for a local population, where mothers raise their young and the reproductive potential of an area is concentrated. Maternity roosts consist mainly of females and young bats. However, depending on the roost type and possibly the tradition of a local population, approximately 20% males can be present in maternity roosts (Issel 1950; Gaisler 1963a; Frühstück 2005). The number of males present in maternity roosts in Austria differed between roosts and between controls. A colony in a hollow concrete bridge had a proportion of over 30 % adult males present, whilst in colonies roosting in attics in the same area, only 10-15 % males were captured when emerging (Frühstück 2005). Recently, Zarzoso-Lacoste et al. (2017) used non-invasive genetic methods to examine the sex ratio of adult lesser horseshoe bats at 19 maternity roosts in northern France. Using faecal DNA collected from these roosts to perform genotyping and sex determination analysis, they found that the overall proportion of males present was 26%, but ranged from 0-50%. Using a purely acoustic method, Lehnen et al. (2018) determined the sex-ratio in four colonies in Germany, and found a proportion of adult males up to 63%.

Maternity roosts of lesser horseshoe bats typically offer a variety of temperatures (Gaisler 1963a; Schofield 1996; Reiter et al. 2004). Thus, there may be areas within the roost which reach high temperatures, albeit with a higher variation in temperatures too. On the other hand, there may be areas with more stable and also lower temperatures, which are often used during times when individuals use torpor as an energy saving strategy (Gaisler 1963a; Reiter et al. 2004). Seckerdieck et al. (2004) described the use of a cooler alternative roost (beer cellar) by a maternity colony in Thuringia, Germany, during periods of cooler weather.

Colonies in Britain and probably in other areas use satellite roosts in order to minimise flight distances to foraging areas (Schofield 1996). Satellite roosts are alternative day roosts in the vicinity of maternity colonies (Kayıkçioğlu and Zahn 2002). The number of bats using this roost type can vary greatly, with individual or small clusters of bats being present one day but gone the next (Schofield 2008; Kayıkçioğlu and Zahn 2002). In a study in Wales, female bats carrying young were present during the day in satellite roosts, but no pups were left overnight (Schofield 2008). In Bavaria Kayıkçioğlu and Zahn (2002) found that temperature was an important factor for roost switching and they occasionally even found non-volant juveniles in satellite roosts.

Solitary male and female *R. hipposideros* can be found in a great number of different roost types including buildings, bridges, caves, crevices in cliffs and others. Up to a few individuals will be found in these roosts and typically no young are present (Reiter et al. 2004; Dietz et al. 2016).

In addition to their day roosts, lesser horseshoe bats make extensive use of night roosts (Schofield 2008; Knight and Jones 2009; Downs et al. 2016b). In their study of three colonies of *R. hipposideros* in Britain, more than 75% of bats used night roosts away from the maternity roost, typically in buildings (Knight and Jones, 2009). Similar results were obtained by Downs et al. (2016b).

Up to five different night roosts were used by individual bats, with the number of night roosts correlated with home range and core area size. Night roosts were significantly nearer to core foraging areas than were maternity roosts, with 64 to 86% contained within core nuclei. Thus, Knight and Jones (2009) postulate that minimisation of distance to feeding sites may be the

primary function of night roosts, with roosts being used for resting and digestion between foraging bouts. Downs et al. (2016b) reported a night roost (a heated boiler room) that was used by 12 out of 15 radio-tracked bats, indicating that the bats were making use of the warmer temperatures or were using the site for social purposes such as information transfer among relatives.

A great variety of man-made structures are used as night roosts, but underground sites and even trees were also found to serve as night roosts (Knight and Jones 2009; Downs et al. 2016b; Dietz et al. 2016)

Lesser horseshoe bats may not switch directly between maternity roosts and hibernation roosts but may make use of transitional roosts (Gaisler 1963a, Schofield 2008). Transitional roosts are typically used in April and October and bats are usually found in torpor (Schofield 2008). For some individuals transitional roosts may also serve as hibernacula (Gaisler 1963a).

Lesser horseshoe bats use a variety of different roosts types to hibernate. Predominantly hibernating in caves, this species now uses a variety of secondary roost types like abandoned mines, cellars, abandoned tunnels or bunkers as hibernacula (Issel 1950; Gaisler, 1963a; Dietz et al. 2016). The thermopreferendum of *R. hipposideros* during hibernation was studied under natural conditions (Gaisler 1963a and 1970) and in the laboratory combined with measurements in the hibernacula (Harmata 1969). This species is often found hibernating at temperatures of 7-8°C (Harmata 1969), with temperatures ranging from 2-12°C (Gaisler 1963a; Harmata 1969; Gaisler 1970; Kokurewicz and Kováts 1989). Variation and the temperature ranges used by individuals might reflect geographical differences (Kokurewicz and Kováts 1989). As a consequence of the preferred temperatures, lesser horseshoe bats are found in parts of caves and other underground sites with warmer and more stable microclimatic conditions compared to some other bat species (Gaisler 1963a; Kovarik 1997, Zukal et al. 2005).

Mating of lesser horseshoe bats often takes place at underground sites or at hibernacula (Gaisler et al. 2011; Dietz et al. 2016).

### **Foraging areas**

Lesser horseshoe bats are often found in lowlands, but also in highlands and in areas with mild climatic conditions up to 2000 m.s.l. (Dietz et al. 2016). Furthermore, habitats of lesser horseshoe bats are characterised by a great structural diversity (Bontadina et al. 2006; Dietz et al. 2016).

Woodland was identified as the key foraging habitat of *R. hipposideros*. Reiter (2004b) found that woodland was more abundant around existing maternity roosts compared to randomly selected churches and castles and that colony size increased with the proportion of surrounding woodland. Boughey et al. (2011) found a strong correlation between lesser horseshoe bats maternity roosts and the proximity of broadleaved forests.

Radio-tracking studies from Austria (Reiter et al. 2013), Belgium (Motte and Libois 2002), Germany (Zahn et al. 2007, Frank et al. 2016), Poland (Kokurewicz et al. 2008), Britain (Bontadina et al., 2002; Schofield et al. 2002; Downs et al. 2016a) and Switzerland (Bontadina et al. 2006) confirmed woodland as the most important foraging habitat for this species.

According to several studies *R. hipposideros* preferred broadleaved woodland over other woodland types as foraging areas (Bontadina et al. 2002; Biedermann et al. 2004; Kokurewicz et al. 2008; Downs et al. 2016a). In some cases lesser horseshoe bats primarily (Schofield et al. 2002) or partially (Bontadina et al. 2002; Biedermann et al. 2004; Kokurewicz et al. 2008) preferred riparian woodland. In contrast, no clear preference for a particular type of woodland was found by Bontadina et al. (2006) or Zahn et al. (2007). Thus, differing results may reflect the importance of the habitat types available to this species, as well as differences in the characteristics of the same habitat type in different regions. Moreover, Knight (2006) suggested that the level of tree cover and/or the availability of edge habitats may be more closely correlated with the carrying capacity of a given area than the extent of broad-leaved woods.

Despite the fact that woodland is the key foraging habitat in many cases, other habitat types are used by the lesser horseshoe bat as well. Depending on the studied region and the resolution of the radio-tracking data the following habitat types were identified as important foraging areas too: Scrub and shrub (garden) habitats (Downs et al. 2016a; Dietz et al. 2016), parkland habitats (Downs et al. 2016a), hedgerows and green lanes (McAney and Fairley 1988a; Motte and Libois 2002; Schofield et al. 2002, Dietz et al. 2016), isolated trees (McAney and Fairley 1988a; Biedermann et al. 2004; Downs et al. 2016a) and grazed grassland (Downs et al. 2016a).

Information about foraging areas of *R. hipposideros* in southern regions is scarce. A radio-tracking study by I. Dietz (unpubl.) in Bulgaria, revealed, that individuals spent 80% of their time foraging in villages (Dietz et al. 2016). Habitat types used by the bats were gardens rich in vegetation, orchards, hedgerows and grazed pastures (Dietz et al. 2016).

### **Spatial Movement**

The lesser horseshoe bat has extremely short wings, enabling highly manoeuvrable search flight close to vegetation (Dietz et al., 2006). Together with high frequency ultrasound pulses (Ahlén 1988), and the ability to eat without loss of echolocation (Ransome 1991), *R. hipposideros* is well equipped for hunting in dense vegetation.

These morphological adaptation's result in slow flight speed (Bontadina et al. 2006), and hence, most foraging areas found by radio tracking were close to their day roosts (Tab. 1). Mean distances, travelled by individuals to their foraging areas appeared to be very consistent in all studies, and ranged from 1.0 to 1.7 km. However, maximum distances recorded reached more than 4 km, with one individual foraging 4.7 km away from the roost (Reiter et al. 2013; Tab. 1).

At a maternity roost in Wales, UK, fifty per cent of the tracking locations were made within 600 m (Bontadina et al. 2002). Larger distances for fifty percent radio tracking data were reported for two maternity roosts in Austria, with 1172 m and 1241 m, respectively (Reiter et al. 2013).

If the utilization pattern of *R. hipposideros* is compared to a uniform distribution, foraging areas up to 2.3-2.5 km from the roost are used more than expected. This result was obtained in Wales (Bontadina et al. 2002) as well as in Austria (Reiter et al. 2013). Therefore, if radio-tracking data is lacking, this perimeter is considered as suitable distance around maternity

roosts for undertaking conservation measures in foraging areas (Bontadina et al. 2002, Bontadina et al. 2006; Reiter et al. 2013).

**Table 1.** Foraging characteristics of individual *Rhinolophus hipposideros* revealed by radio-tracking. Mean  $\pm$  STD, (Min-Max), n = number of individuals

Study Site	Foraging area (MCP, ha)	Core foraging area (50% kernel, ha)	Mean distance to day roost (km)	Maximum distance to day roost (km)
Carinthia, Austria, REITER et al. 2013	202 $\pm$ 135 (57-441) n = 7	12 $\pm$ 5 (8-22) n = 7	1.3 $\pm$ 0.7 (0.4-2.4) n = 7	2.5 $\pm$ 1.1 (1.2-4) n = 7
Carinthia, Austria, REITER et al. 2013	190 $\pm$ 120 (68-430) n = 7	8 $\pm$ 3 (4-12) n = 7	1.3 $\pm$ 0.4 (0.6-1.9) n = 7	2.8 $\pm$ 1.2 (1.7-4.7) n = 7
Wales, UK, SCHOFIELD et al. 2002	97 $\pm$ 95 (12-353) n = 11	21 $\pm$ 8 (8-35) n = 11	---	1.4 $\pm$ 0.7 (0.4-2.7) n = 11
Sachsen, Germany, BIEDERMANN et al. 2004	240 $\pm$ 170 (114-681) n = 9	---	1.4 $\pm$ 0.5 (0.8-2.5) n = 9	2.4 $\pm$ 0.9 (1.5-4) n = 9
Corse, France, BEUNEUX et al. 2008	933 (603.2-1432.2) n=30	7.5 (3.10-16.9) n=30	1 (0.29-1.8) n=30	2.2 (0.72-1.8) n=30
Lorraine, France, GUILLAUME & ROUE 2008	160 $\pm$ 165 (23.8-736.6) n = 16	10.6 $\pm$ 8.6 (1.1-29.7) n=16	1.6 $\pm$ 1.0 (0.2-3.6) n = 14	1.9 $\pm$ 1.1 (0.3-4.3) n = 14
Bouches-du-Rhône, France, Groupe Chiroptères de Provence 2015	545 (185-1750) n=10	40 --- n=10	1.7 --- n=10	3.0 --- n=10

Lesser horseshoe bats prefer roosts that are close to woodland (Reiter 2004b). However, if the next patches of woodland are somewhat further away, they are frequently observed flying under the cover of the canopy of outgrown hedgerows or along overgrown paths and tracks (Schofield 2008). These commuting routes may be of greater importance in landscapes with lower proportions of woodland available by reducing the predation risk, providing shelter from wind, facilitating the orientation and serving as foraging habitat (Bontadina et al. 2006). By using commuting routes the bats can leave the roosts earlier and thus increase the foraging time (Bontadina et al. 2006; Zahn et al. 2007, Reiter et al. 2008).

Since lesser horseshoe bats avoid open spaces whenever possible they use woodland edges, tree lines, hedges, rocky overhangs, streams, fences, walls or other linear landscape elements for commuting (McAney and Fairley 1988a; Motte and Libois 2002; Zahn et al. 2007; Ramovs et al. 2010; Downs et al. 2016a).

Although, Downs et al. (2016a) recorded commuting *R. hipposideros* mainly along the edges of fields near to hedgerows or woodlands, they identified more open commuting routes too. The longest of these was in excess of 200 m through long-established open parkland during the darkest period of the night. Distances of 30–100 m were also flown between mature parkland trees (Downs et al. 2016a). An extreme example of commuting in open habitats was described by Zahn et al. (2007). They recorded two female *R. hipposideros* flying at least 1.2 km (probably 1.5 km) from an island across a lake on separate occasions. One individual roosted on the mainland for two days, the other bat left the island and returned on the same night (Zahn et al. 2007).

This species is typically found at lower altitudes (Spitzenberger and Bauer 2001; Dietz et al. 2016). However, maternity roosts of this species are reported from altitudes up to 1.200 m.s.l. in Austria (Reiter 2002), and 1.400 m.s.l. in Switzerland (Stutz 1989; Lutz Mühlethaler and Bader 2021). In France maternity roosts in buildings are even known up to 1.800 m.s.l. for example from the Provence-Alpes-Côte d'Azur (Drousie and Cosson (2016) and summer and winter records are reported up to 1.800 m.s.l. from the Haute-Savoie department (Groupe Chiroptères de la LPO Rhône-Alpes 2014). Hibernacula are found at the same height in Austria (Spitzenberger and Bauer 2001). However, Roer and Schober (2001) and Dietz et al. (2016) indicate 2.000 m.s.l. as the known maximum altitudes with records for this species, but without further details.

The lesser horseshoe bat is a sedentary species and migration distances between maternity roosts and hibernacula are usually less than 20 km (Issel 1950; Abel 1960; Kepka 1960; Mrkos 1960; Hutterer et al. 2005). However, Kepka (1960) and Mrkos (1960) observed regular movements from 20 km up to 50 km in Austria. The longest movements recorded were: 153 km (Heymer 1964) in France, 150 km in the Netherlands and Belgium (Bels 1952) and 146 km in Poland (Harmata 1992).

The direction of the movements are usually irregular (Gaisler and Hanák 1969), but Kepka (1960) found a northwest-southeast direction in south-eastern Austria (Hutterer et al. 2005). And according to Hanák et al. (1962) adult males undertake longer flights and are generally more vagrant than adult females and young.

## Diet

The lesser horseshoe bat is a generalist species that feeds on a great number of prey species (McAney and Fairley 1989, Bontadina et al. 2008, Lino et al. 2014, Dietz et al. 2016). Depending on the study area Diptera, Lepidoptera, Trichoptera, Neuroptera, Hymenoptera, Coleoptera and Hemiptera are often the most abundant insect orders found in the faeces or discarded fragments (McAney and Fairley 1989; Beck et al. 1989; Arlettaz et al. 2000; Bontadina et al. 2008; Lino et al. 2014; Mitschunas and Wagner 2015).

Results from a study in a modified Mediterranean landscape in Portugal suggests, that within the broad dietary niche, *R. hipposideros* is not opportunistic, but actively selecting Diptera as a preferred food item (Lino et al. 2014). On the other hand Bontadina et al. (2008) showed that the diet composition of *R. hipposideros* mirrored the local insect prey abundance, confirming an opportunistic foraging strategy in their study in Switzerland. Mitschunas and Wagner (2015) found a seasonal compositional variation in all four annual samples, as well as a compositional variation between samples from different colonies, but not between the two annual samples obtained in consecutive years from the same colony. Differences between colonies appeared at least to some extent to reflect differences in availability of foraging habitats (Mitschunas and Wagner 2015). Very similar prey consumption between years was found by Beck et al. (1989) for a colony in Switzerland, too.

A strong variation between months was typically found in studies with regular dietary analysis during the summer season (McAney and Fairley 1989; Bontadina et al. 2008; Mitschunas and Wagner 2015). However, Lino et al. (2014) found that the arthropod diversity remained fairly constant in the diet, but not in terms of prey availability in Portugal.

Furthermore, despite the marked differences in foraging habitats, the results from their study in the Mediterranean area closely match those of studies done in Western and Central Europe, suggesting that *R. hipposideros* feeds on similar taxa all across Europe (Lino et al. 2014). A similar result with Diptera, Lepidoptera and Hemiptera as the most important prey orders was found by Ahmim and Moali (2013) in a mountainous area with mostly open habitats in Algeria and by Bono and Toffoli (2016) in the Italian Alps. Feldman et al. (2000) identified almost the same insect orders from faeces collected from bats mist netted at the Dead Sea region, Israel. However, Dietz et al. (2016) reported that the diet of *R. h. midas* nearly exclusively consist of flying ants in semi-desert areas, at least seasonally (see also Feldman et al. 2000).

McAney and Fairley (1989) compared the diet of four maternity roosts and four male roosts in Ireland. But they failed to detect any differences between females and males concerning their prey. Williams et al. (2011) studied the winter diet across the British and Irish range of the lesser horseshoe bat. Differences in the dietary composition probably relate to local habitat differences. Nevertheless, across sites Tipulidae/Trichoceridae were always the most frequently eaten prey in winter, with Mycetophilidae and Anisopodidae also eaten frequently (Williams et al. 2011). The ecology of the important prey families indicated the value of damp woodland with decaying wood and grazing animals, particularly cattle, for the winter foraging of this species (Williams et al. 2011).

By employing a dual-primer DNA metabarcoding analysis of DNA extracted from faeces collected in three colonies in a wine region in Rioja, Spain, Baroja et al. (2019) studied the consumption of pest species by *R. hipposideros*. Overall, 395 arthropod prey species



belonging to 11 orders were detected, and lepidopterans and dipterans were the most diverse orders in terms of species (Baroja et al. 2019). Altogether, 55 pest species were identified, 25 of which are known to cause significant agricultural damage and eight are regarded as pests affecting grapevines. The composition of pest species in faeces changed significantly with the season (Baroja et al. 2019). And Kayıkçıoğlu and Zahn (2005) registered high proportions of Culicidae and Chironomidae in the faeces of lesser horseshoe bats from a roost at an island in the lake Chiemsee, Bavaria.

## Behaviour

### Foraging Behaviour

Early studies of the overnight activity failed to describe any overall pattern (Gaisler 1963c; McAney and Fairley 1988b). However, Schofield (1996) reported frequent peaks in activity at dusk and dawn.

More detailed information could be obtained from radio-tracking studies, where several activity bouts during the night could be registered (Knight and Jones 2009; Downs et al. 2016b). The number of these bouts per individual varied between the studies. Downs et al. (2016b) found a mean number of activity bouts per night of 3.5, each lasting for an average of 148 minutes. But they also noted that females had longer median bout durations than males (Downs et al. 2016b).

In their study in Gloucestershire, UK, Downs et al. (2016b) reported that an average of 3.3 night roosts were used per bat during a night of foraging. A lower number of night roosts per bat were reported by Knight and Jones (2009) at three roosts in Britain, ranging from 1.3-1.8 roosts per night.

Direct observations of hunting lesser horseshoe bats are difficult to obtain, especially in woodland (Bontadina et al. 2016). But infrared-video recordings and direct observations under favourable conditions show very similar results: *R. hipposideros* often hunts in circles and loops within the canopy or between the branches of trees but also at lower heights (Jones and Rayner 1989) and sometimes even from the ground (Jones and Rayner 1989). Insects are typically captured and consumed in flight, sometimes using their wings for prey capture (McAney and Fairley 1988a; Jones and Rayner 1989; Bontadina et al. 2016). After Jones and Rayner (1989), lesser horseshoe bats are also able to glean prey from the vegetation. Perch hunting, a foraging behaviour common in *R. ferrumequinum*, is described by Schofield (1996) for lesser horseshoe bats, too. However, this foraging behaviour seems to be rare in *R. hipposideros* and is probably restricted to special circumstances, like late pregnancy. Larger prey is sometimes consumed while perched at a roost or on a branch (Zahn et al. 2007; Bontadina et al. 2016).

Lesser horseshoe bats forage even under rainy conditions (Holzhaider et al. 2002). But colder temperatures and increasing rainfall results in bats flying for longer than usual, presumably due to reduced quality of foraging, whereas wind speed affected *R. hipposideros* differently at different stages of the breeding cycle. Moreover, heavy rain caused a reduction in the duration of the foraging period in several studies (Gaisler 1963c; McAney and Fairley 1988b; Schofield 1996).

The available habitat surrounding the roosts has only a small influence on the foraging behaviour of this species (Reiter et al. 2013). For example, foraging behaviour and spatial

movements of lesser horseshoe bats did not appear to be influenced by fragmentation or other differences in the available habitat (Reiter et al. 2013). When comparing highly fragmented and less fragmented landscapes, Reiter et al. (2013) found no differences in the size of individual foraging areas, or core foraging areas nor the maximum distance travelled by the lesser horseshoe bats. This suggests that the behavioural or ecomorphological constraints probably limit how well this species can adapt its foraging behaviour in degraded landscapes (Reiter et al. 2013). However, the individual foraging areas were more clustered in a highly fragmented landscape and the overall MCP of all bats was greater compared to roosts located in a heavily wooded area (Reiter et al. 2013).

### **Emergence and Commuting Behaviour**

Before leaving their roosts, lesser horseshoe bats perform short excursion flights immediately outside roost entrances. This behaviour is repeated by a smaller proportion of the colony at dawn as the bats return to their day roost. This has been attributed to a light sampling behaviour of originally cave dwelling species (Gaisler et al 1963c; Schofield 1996) and is a means of the species regulating its circadian rhythms. However, Schofield (1996) occasionally observed a similar behaviour in the middle of the night, suggesting that it may also serve as environmental testing for temperature and precipitation.

Though accounting only for a very short proportion of the daily time budget, the nightly emergence from the roost can have a critical impact on the fitness of individual bats (Duvergé et al. 2000). Overall, sunset had the strongest influence on the emergence time of lesser horseshoe bats, as is the case in other bat species too (Schofield 1996; Reiter et al. 2008; Ramovs et al. 2010; Warchałowski and Pietraszko 2019). The same was found by Meyer (2000) for the return times in the morning which were correlated with sunrise.

Emergence times reported from studies in Central Europe are typically between 20-30 minutes after sunset (Reiter et al. 2008; Warchałowski and Pietraszko 2019). And Downs et al. (2016b) reported a mean emergence time of 29 minutes after sunset from Gloucestershire (UK), which fits well with the results from Mainland Europe. Moreover, they found a difference in the median emergence time between males and females, with females having an earlier emergence time compared to males (Downs et al. 2016b).

Additional factors modify the exact timing of the nightly emergence, such as: weather (McAney & Fairley 1988b, Reiter et al. 2008), reproductive state of the females (Reiter et al. 2008), distance to the next woodland patches (Reiter 2002), and external illumination. Moreover, installing artificial hedgerows for optimising emergence routes from the roosts in Austria resulted in earlier emergence (Reiter et al. 2008).

### **Social Organization**

Most individuals of this species live in colonies during summer, consisting predominantly of females, but with males being present in different and varying proportions (see Roosts). It is important to note, that the role and importance of the varying number of males within the maternity roosts is unclear so far.

The most important function of the colonies is the social thermoregulation, whereby females (and males) form clusters when ambient temperatures are low and are hang more solitary when temperatures are increasing (Schofield 1996, Reiter 2002 also see Physiology).

Schofield (1996) found a temperature of 30.9°C in the cluster, which reflects the optimal temperature for this species during activity.

Bats are typically found clustering at temperatures below 22°C in Austria and hanging predominantly solitary at temperatures above 24 °C (Reiter 2002). Occasionally clusters were formed at higher temperatures too (Reiter 2002).

The forming of a cluster and the dynamics within a cluster was studied by means of infrared video recordings in Austria (Dirnberger 2016) and proved to be a surprisingly dynamic process. Clusters typically build up steadily. On average, 50 % of individuals joined the cluster after 89 minutes, and 90% of individuals after 331 minutes. The maximum cluster size was reached on average 588 min after the initial cluster formation began (Dirnberger 2016). Usually the colony forms one big primary cluster, but in the first hours often one additional smaller cluster was noticeable too. Whilst the cluster are building up a constant turnover of individuals is reported by Dirnberger (2016), with bats joining the cluster but also leaving the cluster too. After the cluster has reached its final size, there is still a constant turnover with 4-7 individuals joining and leaving the cluster during 5 Minute-Intervals (Dirnberger 2016). Grooming and urinating were identified as reasons for leaving the cluster.

Lesser horseshoe bats typically land near the cluster and subsequently move to the edge of the cluster to join in, whilst leaving the cluster also occurs from inside the cluster (Dirnberger 2016). Overall, this behaviour resulted in no fixed positions of single individuals within the cluster or at the edge of the cluster (Dirnberger 2016), but in a dynamic system in space and time.

### Home Range

Radio-tracked females exploited hunting territories, expressed as Minimum Convex Polygon (MCP), within a great range (Tab. 1). Mean size of MCPs were quite similar in Austria (Reiter et al. 2013), Germany (Biedermann et al. 2004) and Lorraine, France (Guillaume and Roue 2008), ranging from 160-240 ha. However, individual variation was noticeable, which could also be an effect of incomplete MCP's for some individuals (Tab. 1). Smaller foraging areas were reported from Wales, UK, by Schofield (2002) with 97 ha. And very great hunting areas were registered in southern parts of France, in Corsica with 933 ha (Beuneux et al. 2008) and in Bouches-du-Rhône (Groupe Chiroptères de Provence 2015) with 545 ha.

A female *R. hipposideros* studied in Switzerland (Bontadina et al. 2006) hunted several hours in steep forests, at dawn it flew to the highest parts of the forest at over 1.500 m.s.l. before it returned to the roost 800 m lower.

### Echolocation

The lesser horseshoe bat is the smallest species of the Rhinolophidae in Europe and has one of the highest echolocation frequencies of any European bat species (Kay and Pickvance 1963; Ahlén 1988; Schofield 1996). In the first studies the frequency of the calls was measured between 110-114 kHz, with a mean frequency of 112 kHz (Kay and Pickvance 1963) and Ahlén (1988) found that the call duration was about 50 ms with a pulse repetition rate of about 10 per second. Calls consist of a CF segment, the strongest component of which was at 110 kHz, with an upward FM sweep at the beginning and a terminal downward sweep (Ahlén 1988). Interestingly, this is the second harmonic, while there is a weaker first harmonic at about 55 kHz (Ahlén 1988). Jones and Rayner (1989) noted that 83% of the calls

included FM information, and that the downward sweep at the end had a greater bandwidth than the upward sweep at the beginning of the call in 77% of the calls.

Jones et al. (1992) investigated the variation in the calls of different sexes and ages of *R. hipposideros* and found that females had a higher call frequency than males and bats older than one year have calls higher in frequency than those produced by animals under one year. Furthermore, Kay and Pickvance (1963) reported that the call frequency increased from 30 to 100 kHz in pups between one to two weeks old. But, within sexes, frequency is not correlated with forearm length and it is important to note, that because males are smaller than females one would expect that males have higher calls compared to females due to morphological dependences.

The difference in call frequency between the sexes can be used to discriminate between males and females based on their calls in the field (Jones et al. 1992; Frühstück 2005; Lehen et al. 2018). By using Approximate Bayesian Computation (ABC) Lehen et al. (2018) developed a method to infer sex ratio of colonies from acoustic data.

Sex and age differences in call frequencies may have several implications (Jones et al. 1992): i) calls may provide information about sex and age, and thus, being important in communication, ii) a large variation in calls among individuals may facilitate recognition of an individual bat's own echoes, and iii) higher frequencies are better at resolving small targets and may influence prey size selection.

By comparing published resting frequencies of handheld individuals, a regional variation in the call frequencies used by *R. hipposideros* is evident, even if available for only a few regions (Frühstück 2005; Russo et al. 2007; Dool et al. 2016b; Tab. 2). Compared to the call frequencies in Ireland, the UK and Italy, lesser horseshoe bats tend to use lower calls in Austria (Tab. 2). Furthermore, a marked difference in the call frequencies was found between this species on Sardinia and the Italian Peninsula, with very high call frequencies in Sardinia (Russo et al. 2007; Tab. 2). Species recognition and facilitation of intraspecific communication were thought to be factors for this phenomenon (Russo et al. 2007).

However, it is important to note, that it takes several calls until the resting frequency is reached (Siemers et al. 2005) and, thus, measurements have to be standardised when comparing different regions in future studies.

**Table 2.** Call frequencies of individual handheld *Rhinolophus hipposideros*. Mean  $\pm$  STD, (Min-Max), n = number of individuals

Study site	Frequency adult females	Frequency adult males	Frequency juvenile females	Frequency juvenile males
Ireland, Dool et al. (2016b)	112.2 (105.3-115.0) n = 143	108.9 (105.7-113.8) n = 106	111.6 (108.2-113.4) n = 59	108.6 (106.2-112.0) n = 69
UK, Jones et al. (1992)	114.1 ± 1.5 ---- n = 12	111.2 ± 0.7 ---- n = 12	113.0 ± 1.9 ---- n = 8	110.5 ± 0.6 ---- n = 9
Austria, Frühstück (2005)	110.2 ± 1.4 ---- n = 16	106.3 ± 1.2 ---- n = 4	109.6 ± 1.6 ---- n = 9	105.6 ± 0 ---- n = 1
Sardina, Russo et al. (2007)	117.2 ± 1.28 ---- n = 23	113.5 ± 1.03 ---- n = 25	----	----
Peninsular Italy, Russo et al. (2007)	113.3 ± 1.24 ---- n = 21	110.6 ± 0.66 ---- n = 5	----	----

Like in other bat species, lesser horseshoe bats use feeding buzzes in the late phase, when approaching their prey (Schofield 1996). The feeding buzz of *R. hipposideros* is characterised by a distinct and sudden increase in the pulse repetition rate, a decrease in call duration and an increase in the presence of FM sweeps (Schofield 1996).

Different social calls of this species were described by Andrews et al. (2017) in the UK and Dirnberger (2016) in Austria. Andrews et al. (2017) described ultrasonic calls with fundamental frequencies between 15–42 kHz from seven categories of infant development calls and 15 categories of adult ultrasonic social calls according to the frequency and duration of the fundamental, the number of harmonics and the number of calls in a sequence. These include distinctive polyharmonic isolation calls of newborn *R. hipposideros* (Andrews et al. 2017).

Furthermore trill advertisement calls in the hibernaculum were related to mating behaviour (Andrews et al. 2017).

## Reproductive Behaviour

Surprisingly little is known about the mating system and social organisation of *R. hipposideros* during the mating period. Kolb (1950) reported some details of mating behaviour from attics in Germany. Individuals were described as being “very lively”, with close hovering flights in pairs, biting behaviour and the production of social calls noted (Kolb 1950). Gaisler et al. (2011) found a mating pair in a cave in the Czech Republic. The bats were found in dorso-ventral contact and not being wrapped in their wing membranes. The male had bitten his mate’s dorsal fur and the lower bat, evidently a female had raised its head and moved it slightly as when echolocating (Gaisler et al. 2011).

So far, no typical autumn swarming behaviour is reported for the lesser horseshoe bat. However, Davies and Thomas (2017) described a spring swarming behaviour for this species from Wales, UK. Besides a spring peak in social activity at caves, which are not known to be used for hibernation, they also noted well-developed epididymides, indicating that the males were in reproductive condition (Davies and Thomas 2017).

## Parasites and Diseases

Lesser horseshoe bats are frequently parasitised by the long-legged bat tick, *Ixodes vespertilionis*, which may occur in large numbers in roosts in both caves and buildings (Piksa et al. 2013). It may also be parasitised by the tick *I. simplex* and the flea *Rhinolophopsylla unipectinata*, and is known to be a host to the Nycteribiid flies, *Phthiridium biarticulatum* and *Nycteribia schmidlii* (Ševčík et al. 2011, Burazerović et al. 2018). A number of mite species have been recorded to date: *Macronyssus uncinatus*, *M. rhinolophi*, *M. ellipticus*, *Eyndhovenia euryalis*, *Paraperiglischrus rhinolophinus*, *Neomyobia chiropteralis*, *Steatonyssus spinosus*, and *Spinturnix myoti* (Baker and Craven 2003, Stanyukovich 1997, Dool et al. 2018). It is furthermore host to the protozoan parasite *Eimeria hessei* (Afonso et al. 2016). Due to the high level of interest in bats by virologists, a range of viral taxa have also been reported for the lesser horseshoe bat (summarised in the bat viral database, DBatVir). However, many of these records are clear misidentifications of the bat host as they occur well outside the known range for this species (e.g. in Yunnan province China). Unless the species identification was confirmed by a suitably experienced bat worker or by other means (e.g. genetics), it thus seems prudent to be sceptical of such reports.

## Population Ecology

### Population dynamics

#### 1. Population declines during the 1900’s

Dramatic declines of the lesser horseshoe bat occurred in several European countries starting in the 1940s and continuing until the 1980’s, including Switzerland, Germany, Poland, Netherlands, Belgium, Luxembourg, Ukraine and in regions of France (Pas-de-Calais, and Ile-de-France) (Bontadina et al., 2008 and references therein, 2000; Dubie and Schwaab, 1997; Kokurewicz, 1990; Kovalyova, 1997; Stebbings, 1988). The species is still extinct in the Netherlands, and remains relatively rare in Belgium. These declines resulted in a retraction of the species distribution along part of the northern limit. Pronounced declines were not reported in Ireland (McAney 1997) which is also part of the northern distributional limit. However, a population genetic study conducted for this species in Ireland found evidence for historic declines of 3-37% in 31 out of 37 colonies studied (Dool et al., 2016b).



A number of hypotheses have been put forward to account for these declines: shortage of prey, inter-specific competition prey, the application of pesticides including DDT, intensive agricultural practices and the destruction or loss of roosting sites (e.g. Arlettaz et al., 2000; Bontadina et al., 2000; Fairon, 1997). Prey shortage and competition are more or less ruled out and loss of woodland per se does not appear to be a sufficient explanation (Bontadina et al., 2008). The definitive causes(s) of these declines remain unresolved.

One difficulty is that, despite long-term ringing or monitoring activities in some regions (e.g. Czech republic since late 1940's, France, after 1945), detailed monitoring programs did not exist in a consistent manner across the species distribution (or for bats generally). Therefore, historic data presenting detailed and precise quantification of population numbers over time is rare (e.g. date, locality, number of individuals for each site monitored). This raw data is needed to interpret % declines, or general statements of decline/colony loss which are typically reported (Stebbing, 1988). It is possible for example, that this species declined across its distribution, but that this was only readily visible in the parts of the northern range limit where populations may have been small and rather isolated.

Additionally, it is important to note that declines in several other bat species were reported concurrently with that of the lesser horseshoe bat, in the same regions. For example, in the Netherlands severe declines in many species were reported, including *Myotis emarginatus*, *M. myotis*, *M. mystacinus/brandtii*, *M. dasycneme*, *Plecotus* spp. and *Rhinolophus ferrumequinum* (Stebbing, 1988).

A number of factors would render declines more obvious: e.g. conspicuous species (e.g. *R. hipposideros*), small colonies at low densities, and the existence of some level of monitoring. Therefore, it is again possible that further species were also declining but that this was never recorded. Levels of decline may have also varied regionally. The causes of the declines remain conjectural, but their scale justifies that this question be revisited. New tools exist to help decipher the principal cause, including fine-scale habitat modelling, population genetic methods which can uncover and date changes in population sizes and tests for chemicals and pesticides in museum specimens or guano deposits. It would be prudent to include all bat species across Europe as detailed historic monitoring data does not exist to prove that declines were not more widespread geographically and taxonomically.

## 2. Recent population increases or recoveries

Increases in lesser horseshoe bat populations have been reported from several countries, or regions during the most recent decades. The trends were usually based on hibernacula counts but also include maternity colonies. Increasing trends have been reported from: several regions of the Czech republic (~1999-2009, Bufka et al., 2009; 1968-2012, Chytil and Gaisler, 2012; 1985-1995, Gaisler, 1997; 1977-2018, Martínková et al., 2020), across the species distribution in Ireland (2007-2015, Roche et al., 2015), in Thuringia Germany (Tress et al., 2012), in Styria Austria (1973-2010 Sackl et al., 2011), in Liguria Italy (2000-2018, Toffoli and Calvini, 2019), the Polish Sudetes (1994-2006, Furmankiewicz et al., 2007), and Western Carpathians in Slovakia (1992-2009, Uhrin et al., 2010). Van der Meij et al. (2015) aggregated monitoring data (1993-2011) for 16 European bat species from 9 countries to assess long-term global trends. Nine of the species showed increases, including the lesser horseshoe bat, with only one species decreasing. However, there were profound differences between biogeographic regions and countries. Further data including more countries and

species is needed to examine whether these trends hold more generally. The authors made the important point (as did Uhrin et al., 2010) that there is no way of knowing whether these increases are simply the slow and ongoing recovery of populations following the strong declines half a century ago.

Additionally, whilst it can be assumed that historic threats including large-scale habitat destruction and deliberate persecution are now greatly reduced due to European environmental protections, there are now new threats facing bats: increasing traffic levels on roads, wind turbine collisions, climate change, light pollution, decreasing insect abundance and the continued use of neonicotinoids (Goulson, 2019; Grilo et al., 2020; Hallmann et al., 2017; Medinas et al., 2021; Mineau and Callaghan, 2018; Van der Meij et al., 2015).

### 3. Factors known to affect population demography

In most monitoring studies, the underlying causes of population declines or increases (see above) are unknown. However, this question has been addressed using direct and detailed monitoring, modelling and by non-invasive CMR techniques. The effects of roost temperature on the timing of birth and growth-rate of lesser horseshoe bat pups was studied in three maternity colonies in Austria, over 2 years (Reiter, 2004b). It was found that lower temperatures in June both delayed and prolonged the birthing period and that inclement summer weather (wet and cool) also resulted in slower rates of growth in juveniles. It has further been shown that climatic conditions occurring over even very short time frames (~ a month) can have strong impacts on lesser horseshoe bat population dynamics (Jan et al., 2017). Detailed climatic information was used in a modelling approach along with 15 years of monitoring data at 94 maternity colonies in Brittany France. October precipitation was found to have a negative impact on colony sizes, whereas June precipitation had a positive impact. Surprisingly, fecundity was also influenced by precipitation, but in an opposing manner to colony size: October precipitation had a positive effect, whilst April rain had a negative impact. These results illustrate the importance of using fine-scale temporal resolutions to understand how bats respond to climatic changes and suggest that complex patterns of co-variation may be occurring which are currently poorly understood (Jan et al., 2017).

The factors impacting population growth rates in *R. hipposideros* were further investigated by Jan et al. (2019a) combining non-invasive CMR and a novel integrated population model (IPM). The authors sampled guano from 35 colonies, 16 from a non-expanding population in Picardy France, and 19 from an expanding population in Thuringia Germany. Collections were made before and after parturition during three years. CMR data were obtained after DNA extraction and genotyping. The IPM was then used to estimate demographic parameters for the lesser horseshoe bat from the CMR data (adult survival, fecundity, juvenile survival), and to infer parentage assignment and abundance data. The range expansion in the Thuringia population was associated with higher net reproduction. Juvenile survival was the main driver of this observed reproduction increase and it is inferred that this was achieved by higher resource acquisition in the expanding population. High resource acquisition may be possible due to relaxed density dependence at the expansion front, although roost and habitat availability and quality could also play a role (Jan et al., 2019a).

Individual-based studies (e.g. radio tracking) and presence-absence data have shown that broadleaf woodland is the preferred foraging habitat for this species and strongly predicts the

presence and size of colonies (Bontadina et al., 2002; Reiter, 2004a; Reiter et al., 2013; Tournant et al., 2013). The relationship between habitat quality and population dynamics was examined in further detail using detailed monitoring data from 94 colonies of the lesser horseshoe bat in Brittany, France (Jan et al., 2019b). Colony size, fecundity and growth rate were estimated for each colony in 2000-2014. This was combined with CORINE land-cover data and climatic variables in a modelling approach at two spatial scales: 500 m and 2500 m around the roost. Correlations between landcover area and shape and demographic parameters were assessed and showed that the landscape in the 500 m buffer around the colony is crucial for population size and dynamics. Furthermore, the strongly positive influence of broadleaf woodland proportion on bat colony size was confirmed. There was also a positive effect of lakeshores and riverbanks on both colony size and growth rate. These results build upon earlier studies and highlight the sensitivity of this species to deterioration of habitat quality around the colony, whether by urbanisation, agriculture (large open fields of crops) and habitat fragmentation. (Jan et al., 2019b).

Although the demographic effects of road-kill have not yet been investigated, the negative impacts of traffic collisions on such long-lived, slowly reproducing species such as bats can be presumed (e.g. Claireau et al., 2019b). Lesser horseshoe bats in particular are prone to road-kill due to the attenuation of their echolocation calls which necessitates flying close to vegetation or low to the ground. Medinas et al. (2021) studied a 51 km road transect over three years (2009-2011) in southern Portugal. The surveys were conducted once per day in the morning by driving along the road at 20-40 km/h. Therefore, the carcasses found represent a minimum number of casualties. 18 lesser horseshoe bats were found dead over the three years and susceptibility to collisions was especially high in the vicinity of high quality habitats. Considering the limited foraging distance of this species and their slow rate of reproduction, that is a substantial number of bats to die in such a small area (along the transect). If these collision rates are not a rarity, the number of bats being killed on roads needs to be urgently assessed and mitigated using appropriate measures (e.g. Claireau et al., 2019c, 2019a).

### **Climate change biology**

Based on predictive models incorporating IPCC projected climate change patterns and current species distributions, the lesser horseshoe bat (and indeed all five species of *Rhinolophus*) are predicted to experience range expansion in all but the most extreme predicted future climate models (Rebelo et al., 2010). However, climatic envelopes alone were considered in this assessment which do not take into account any biotic interactions (competition, predation etc.) or habitat or roost availability (Rebelo et al., 2010), meaning that these projections may be optimistic (Jones and Rebelo, 2013). For isolated populations, or those potentially restricted by geographic barriers, simply shifting their distribution northward may not be possible and such lineages are at high risk of being lost (e.g. Iberian lineages of the lesser horseshoe bat Dool et al., 2013).

It is further known that climate change has the potential to affect several aspects of bat physiology and phenology (Jones and Rebelo, 2013). Increased temperatures may result in some species living outside their thermal optimum; pregnancy duration and hibernation energetics may be impacted and roost availability may decline with climate-induced habitat changes. With an increase in extreme weather events, mortality rates can be expected to increase due to excessively high temperatures and droughts (Jones and Rebelo, 2013).

Changes in precipitation patterns are already occurring and can also affect species' phenology. Studies documenting the demographic impacts of weather conditions are discussed above.

### **Conservation Status**

The lesser horseshoe bat's range is well beyond the borders of Europe, stretching east to the foothills of the Himalayas and south into Africa. Consequently, it is classified as Least Concern by the IUCN at a global level. However, the species underwent a population collapse in much of its northern, central, and western European range during the mid to late 20<sup>th</sup> Century, as a result of habitat loss, roost disturbance and probably the use of organochlorine pesticides. At a European level as well as in the European Union it is classified Near Threatened but the individual classifications at a country level reflect the impact of the population collapse at a more local scale, it was lost entirely from the Netherlands and in neighbouring Belgium it is Critically Endangered. For much of the rest of Europe it is now Least Concern, following a partial population recovery that started at the end of the 20<sup>th</sup> Century.

The main threats to the species continue to be the loss of habitat and roosts. However, these are being compounded by Increasing levels of urbanisation, light pollution, intensification of agriculture and infra-structure development, all of which are fragmenting its habitat. This is particularly challenging for a species that rarely crosses open spaces and requires a vegetatively well-connected landscape to commute between its roosts and foraging areas. The summer roosts of this species are typically in older buildings. Some of these are being lost simply through dereliction but for others the original purpose is now redundant, and they are being converted to uses that are not compatible with housing a bat roost. Those roosting in historical buildings including churches, are increasingly subject to illumination with artificial lighting at night. The increase in tourism associated with show caves, particularly in the central and southern areas of Europe, have increased disturbance at these sites threatening both summer and winter colonies of the species.

### **Management**

Aside from the obvious maintenance and expansion of woodland, the principal foraging habitat for this species, the wider habitat needs to be permeable at a landscape scale to enable them to commute to these foraging grounds. In areas of more open landscape, this can be achieved by increasing linear landscape features such as tree-lines and hedgerows. The increasing use of artificial lighting at night can act as a barrier to this species, even if the landscape features they need for commuting are present. The creation of dark corridors or more strategic deployment of lighting can help to alleviate this problem. This type of mitigation can also be undertaken at a roost level, with the lighting used on historical buildings being directed away from roost entrance and vegetative cover close to roost buildings.

### **Future Challenges for Research and Management**

Our understanding of the roosting and foraging needs of this species are well established and the techniques needed to maintain and enhance both are well published. However, maintaining and improving landscape permeability for this species against a background of increasing

levels of urbanisation and travel infra-structure development, is a key challenge. Specifically, how we provide lesser horseshoe bats with means of safely crossing road and rail links.

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