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Research Article

The diet of the notch-eared bat (*Myotis emarginatus*) across the Iberian Peninsula analysed by amplicon metabarcoding

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Abstract

Myotis emarginatus is one of the few bats known to feed mostly on spiders. In order to study the importance of this type of prey, we analysed the species' diet in five colonies across the Iberian Peninsula using amplicon metabarcoding in order to describe its composition at the species level, and analyse its geographic variability within the peninsula. We identified 138 prey species, belonging to 11 different arthropod orders. Among them, 45 species of spiders were identified, mostly of the orb-web building guild, as consumed by 82 out of 106 studied bats, corresponding to every colony and season sampled. Besides, lepidopterans and dipterans were also consumed in every colony. Among the latter, the stable fly *Stomoxys calcitrans* was especially important in two of the colonies, showing that *M. emarginatus* can also opportunistically exploit different resources or foraging grounds, such as cattle sheds, which affects the composition of its diet also at ordinal level of prey.

Introduction

Despite their predominantly insectivorous behaviour, most Palearctic bats show a wide variety of trophic strategies. Wing morphology and echolocation characteristics of bats influence the way they interact with the foraging environment, and therefore, the prey they find and consume (Emrich et al., 2014). On top of that, local or seasonal changes in food availability will inevitably affect the final composition of their diet (Kunz et al., 2011). Finally, coexistence with other bat species and the mechanisms driving the specific allocation of resources can also be important factors that shape the trophic niche of bats (Chesson, 2000; Adler et al., 2007; Salsamendi et al., 2012; Viglino et al., 2016; Arrizabalaga-Escudero et al., 2018; Schoeman and Monadjem, 2018).

Amongst the primarily insectivorous European bats, *Myotis emarginatus* is the only one known to feed mostly on spiders (Goiti et al., 2011; Kervyn et al., 2012). Worldwide, a diet based on spiders has only been described in two more bat species: *Myotis keenii* in North America (Burles et al., 2008), and *Kerivoula papuensis* in Australia (Schulz, 2000). Spider consumption on these two species is thought to be linked to foraging in cluttered environments and gleaning over immobile prey. *Myotis emarginatus* shows a similar foraging strategy, favouring cluttered forests or areas of complex vegetation for hunting (Zahn et al., 2010; Goiti et al., 2011; Dekker et al., 2013), but also gleaning flies off the walls of cattle-barns (Krull et al., 1991). The precise mechanism used to catch spiders, however, remains unknown.

Spider consumption by *M. emarginatus* reaches almost 80% of the diet's bulk in meridional populations (Goiti et al., 2011) and in Central Europe (Bauerová, 1986). However, in some colonies in Central and North-western Europe, a diet rich in cattle-flies has also been reported, linked to foraging inside cattle-barns (Beck, 1995; Kervyn et al., 2012). Even in these circumstances, spider consumption still reaches 25% of the total diet (Kervyn et al., 2012). Further, Kervyn et al. (2012) visually identified seven species of spiders in the faeces of *M. emarginatus*, and Galan et al. (2018) listed 16 spider species in the faeces of ten *M. emarginatus* individuals through DNA metabarcoding.

Apart from *M. emarginatus* other European bat species also consume spiders although they are seldom the primary food source. For instance, *Plecotus auritus* and some populations of boreal bats (Razgour et al., 2011; Vesterinen et al., 2018) consume spiders occasionally. Other European bat species, such as *Myotis nattereri* or *Myotis myotis*, prey upon spiders as an alternative food resource in times of lower insect abundance or scarcity of preferred prey (Ramos Pereira et al., 2002; Hope et al., 2014).

Nevertheless, spiders do not represent a single functional prey to bats. A variety of functional groups, or guilds, have been identified according to their own hunting behaviour and web structure (Uetz et al., 1999). Non-web building or wandering spiders, for instance, should be detected on and captured directly from the ground or vegetation by gleaning (Hope et al., 2014). Conversely, web-building spiders could be captured from their webs by gleaning (Kervyn et al., 2012) or hawking (Goiti et al., 2011). Finally, small spider species capable of "ballooning" might also be captured by aerial hawking (Hope et al., 2014). Hence, the functional availability of a given spider species to bats will depend largely on the guild the prey belongs to.

Difficulties to accurately identify arthropod taxa by external morphological traits hamper the listing of the bat's prey at the species level, which, if overcome, would allow describing the functional characteristics and lifestyle of the spider prey. The recent application of molecular methods to diet studies (Pompanon et al., 2012) enables a more complete representation of the taxonomical and functional diversity of

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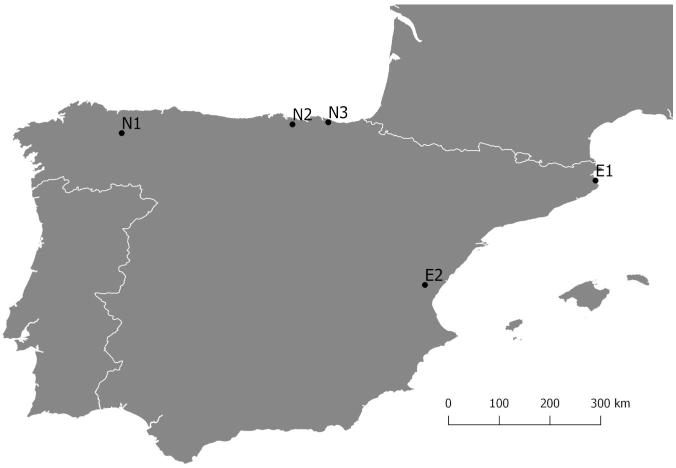

Figure 1 – Sampling sites.

spiders at the species level (Galan et al., 2018). Orb-web building or aerial-web building spiders have been proposed as prey of spider specialist bats (Schulz, 2000; Burles et al., 2009; Goiti et al., 2011), which fits with the species identified in the faeces of *M. emarginatus* so far (Kervyn et al., 2012; Galan et al., 2018).

Our main goals were to assess the importance of spiders in the diet of *M. emarginatus*, as well as their taxonomical and functional diversity, in a large geographical range. In order to do so, we analysed the variability of the dietary composition across five colonies in the Iberian Peninsula. In addition, we analysed seasonal variability in two of the colonies. Previous studies have shown variability in the dietary composition and type of foraging grounds of *M. emarginatus* across locations (Bauerová, 1986; Goiti et al., 2011; Kervyn et al., 2012), which is common in bats, especially at a broad geographical scale (Clare et al., 2014a; Aizpurua et al., 2018). Therefore, covering a large geographical range is important to reflect the most complete niche breadth (Aizpurua et al., 2018).

Methods

Study area

Bats were captured in five different locations along the northern (colonies N1, N2 and N3) and eastern coasts (colonies E1 and E2) of the Iberian Peninsula (Fig. 1), in order to cover a broad geographical range. All the chosen locations are stable breeding colonies for *M. emarginatus* alone or with other bat species, and were previously known by the research team, to ensure that all sampling could be made in a single night fieldwork, therefore avoiding excessive stress on individuals of any species occupying the same roost.

The climate in colonies N2 and N3 is temperate oceanic, due to the proximity to the Atlantic Ocean (AEMET and IMP, 2011, p. 17). N2 is in an area where open pastures for cattle are abundant; while colony N3 is mainly surrounded by conifer plantations. Colony N1, on the other hand, is further from the coast and therefore has a warmer and drier climate, transitional between temperate oceanic and Mediterranean (AEMET and IMP, 2011, p. 17); the area is covered by conifer forests and scrublands. The eastern colonies, E1 and E2, have Mediterranean climate: warm average temperatures with summer droughts (AEMET and IMP, 2011, p. 17), where land use consists mostly of open agricultural areas. Conifer forests are also common near E1.

Sample collection

Each location was sampled on a single night in summer of 2012, and colonies N2 and N3 in spring as well. Bats were captured entering the roost after the foraging bout using a harp trap (Tuttle, 1974) and kept in individual cloth bags until they defecated (maximum 40 min). Afterwards, the bats' sex and age were determined, and the animals were immediately released into the roost. Faeces were frozen within 6 hours. A total of 92 bats were captured during the breeding season of 2012: 12 bats in colony N1, 29 bats in colony N2, 28 bats in colony N3, 17 bats in colony E1 and 6 bats in colony E2. Seven additional individuals from both N2 and N3 were sampled in May of 2012.

Ethics statement

Capture and manipulation of bats were performed according to the guidelines for treatment of animals in research and teaching (Sherwin, 2012), and were approved by the Ethics Committee at the University of the Basque Country (Ref. CEBA/219/2012/GARIN ATORRASAGASTI). In each location, captures were performed under license from the corresponding government agency.

DNA extraction, PCR amplification and sequencing

Each individual bat was treated as a sampling unit (Whitaker et al., 1996). We used 20–40 mg of faeces for DNA extraction using DNeasy PowerSoil Kit (Qiagen, Valencia, CA), following manufacturer instructions with some modifications (Alberdi et al., 2018). The samples and extraction blanks were amplified using primers designed by Gillet (Gillet et al., 2005). These primers are modified at the 5' end, by attaching a unique MID sequence that enables identification of individual samples post-sequencing, and an Ion Torrent adaptor sequence (Clare et al., 2014b). PCR procedures were performed using Hot Star Taq Polimerase kit (Qiagen, Valencia, CA) with 35 cycles. Finally, a total of 106 samples, 10 extraction blanks and 5 PCR blanks were sequenced using Ion Torrent sequencing platform in a single run.

Sequence analysis and library building

DNA sequences were assigned to their respective samples using MIDs, the primers were removed and sequences of adequate length (125–145 bp) were chosen using CUTADAPT (Martin, 2011). Afterwards, a quality filter was applied using USEARCH's *–fastq_filter* command (Edgar, 2010), and following the 'expected error rate threshold' (Edgar and Flyvbjerg, 2014). Remaining sequences were clustered into OTUs at 97% identity threshold and chimeric sequences were removed simultaneously using USEARCH's *–cluster_otus* command. Then, for each sample, those OTUs with frequencies lower than 1% were removed with USEARCH's *–otutab_norm* and *–otutab_trim* commands (Mata et al., 2016). Finally, remaining OTUs were compared against online data base BOLD Systems and GenBank. Only matches with similarity values above 98% (Clare et al., 2014b), and an e-value score lower than 1×10^{-20} (Vesterinen et al., 2013) were considered.

Diet description and analysis

The diet of *M. emarginatus* was analysed using presence/absence data of the prey taxa identified at the species level, in each of the sampling units. Results were expressed in frequency of occurrence (FOO) and per cent of occurrence (POO) of each prey species, family and order for each colony. FOO was calculated as the number of bats in which a given prey was identified, divided by the number of bats examined, and multiplied by 100 (Deagle et al., 2018). On the other hand, POO was calculated by dividing all occurrences of a prey with the total number of occurrences of all prey, and multiplied by 100 (Deagle et al., 2018).

Diversity and richness of each colony was measured using Hill numbers (Hill, 1973). To standardize comparisons between colonies of unequal sample size, we performed comparisons based on sample coverage (Chao and Jost, 2012). `iNEXT` package for R (Hsieh et al., 2016) was used to estimate the diversity of the diet of each population through inter- and extrapolation, as well as the coverage value of each of the samples collected. Then, base-coverage value was established as described in Chao and Jost (2012), and richness values and 95% confidence intervals were calculated for each colony.

Distance matrices were calculated independently for the analysis of summer diet variation and seasonal diet variation in package `vegan` (Oksanen et al., 2018) in R (R Core Team, 2018). We used Jaccard's dissimilarity index for presence/absence data of prey species in each bat individual, and Bray Curtis dissimilarity index for prey family and order frequencies (Legendre and Legendre, 2012) in each bat individual.

Based on these matrices, differences in the diet were tested using adonis with 999 permutations (Manly, 1997) in `vegan` (Oksanen et al., 2018), and pairwise multivariate anova based on 999 permutations, using function pairwise.adonis in package `pairwiseAdonis` (Martinez-Arbizu, 2019). In pairwise comparisons, the *p*-value was corrected using "fdr" method (Benjamini and Hochberg, 1995).

Additionally, Fisher's exact test of independence was performed to analyse differences in the frequency of occurrence of spiders across colonies and seasons. Spider species identified were classified according to their guild or hunting strategy following Uetz et al. (1999) and Roberts (2001). The differences in spider consumption across colonies and season were tested using a similar procedure to that explained above.

Results

Geographical and seasonal variability of the diet

After bioinformatic processing, 288 OTUs were compared against online databases. Among them, four OTUs were identified as belonging to the predator, 159 as potential prey items and 36 as non-prey taxa. The remaining 103 were considered as "not identified" as they did not reach the required identity and e-value threshold. Since some OTUs corresponded to the same taxa, 138 different species or genus were identified as potential prey of *M. emarginatus* (Tab. S1).

The Adonis multivariate test showed differences in the specific composition of the diets between studied summer colonies (F=6.253; d.f.=4; *p*=0.001); nonetheless, differences were not significant between colonies N1 and E2 (F=1.481; d.f.=1; *p*=0.079). Out of the 25 prey species identified in N1, and 19 in E2, both colonies shared seven of them, including the spider *Araneus angulatus*, the River Skater *Aquarius najas* or the Diamondback moth *Plutella xylostela* (Tab. S2). Composition differences between colonies remained significant at the family (F=9.0935; d.f.=4; *p*=0.001) and order (F=12.366; d.f.=4; *p*=0.001) level, and in the last case, differences between colonies N1 and E1 were no longer significant (F=1.647; d.f.=1; *p*=0.212).

Overall, in colonies N1, E1 and E2, spiders and moths comprised more than 56% of the occurrences (Fig. 2). In colonies N1, E1 and E2, spiders were the most commonly consumed prey order (N1: 10 out of 12 bats, POO: 45.2%; E1: 17 out of 17 bats, POO: 58.3% ; E2: 4 out of 6 bats, POO: 21.7%), followed by moths (N1: 9 out of 12 bats, POO: 33.3%; E1: 8 out of 17 bats, POO: 18.7%; E2: 3 out of 6 bats, POO: 34.8%), dipterans (N1: 4 out of 12 bats, POO: 9.5%; E1: 8 out of 17 bats, POO: 18.7%; E2: 1 out of 6 bats, POO: 8.7%) and hemipterans (N1: 3 out of 12 bats, POO%: 7.1; E1: 1 out of 8 bats, POO: 2.1%; E2: 2 out of 6 bats, POO: 13.0%). Additionally, bats in colony E2 also consumed odonates (2 out of 6 bats, POO: 13.0%). Finally, coleopterans, neuropterans, plecopterans and hymenopterans were occasionally consumed (POO: <5%) to complete their diet (Fig. 2; Tab. S2).

In colonies N2 and N3 spiders were not the most commonly identified prey order (N2: 18 out of 29 bats, POO: 18.6%; N3: 19 out of 28 bats, POO: 30.5%). Instead, dipterans were the most frequent prey order (N2: 29 out of 29 bats, POO: 66.2%; N3: 22 out of 28 bats, POO: 35.1%). In both cases, the most frequent prey item was the stable fly *S. calcitrans*, which appeared in 96.4% and 68.0% of the samples in these colonies. Additionally, in colony N2 flies of the genus *Musca* reached frequency of occurrence of 89.2%. Besides, in colony N3, beetles were consumed almost as commonly as spiders (19 out of 28 samples; POO: 15.2%). Fourteen of those occurrences belonged to the cerambycid species *Arhopalus rusticus*. In the case of moths, POO was lower than 10% (N2: 9 out of 29 bats, POO: 7.6%; N3: 10 out of 28 bats, POO: 9.1%). Other prey orders identified include Ephemeroptera, Hymeoptera, Neuroptera, Plecoptera, Psocodea, Trichoptera, Mesostigmata and Trombidiformes, none of which reached a POO of 5% (Fig. 2; Tab. S2).

Conversely, the diet composition at the species level did not differ between the two spring colonies (F=1.397; d.f.=1; *p*=0.103). Results were similar when prey items were grouped at the family level (F=0.984; d.f.=1; *p*=0.459) and order level (F=2.318; d.f.=1; *p*=0.158). Spider DNA was found in all individuals of the spring sample, and its POO was 63.3% (N2) and 41.9% (N3). In both cases dipterans (POO: 20% and 25.6% respectively), and lepidopterans (13.3% and 27.9%) completed most of the diet. In both locations significant differences were found between seasons (F=6.027; d.f.=1; *p*=0.001). Overall consumption of dipterans also dropped in spring (N2: 4 out of 7 bats, POO: 20%; N3: 6 out of 7 bats, POO: 25.5%), as *S. calcitrans* was identified in less than 43% of bats. Besides, in colony N3 no *A. rusticus* was consumed during the spring; and in colony N2, none of the bats consumed *Musca* sp. On the contrary, consumption of spiders increased (N2: 7 out of 7 bats, POO: 63.3%; N3: 7 out of 7 bats, POO: 41.8%).

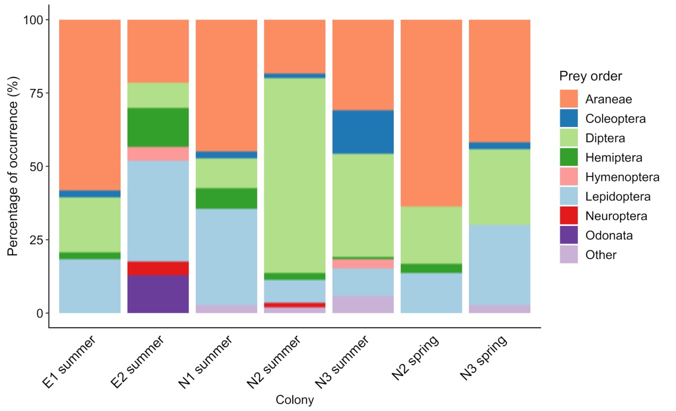

Figure 2 – Diet composition of *M. emarginatus* at the ordinal level by colony and season, as per cent of occurrence. "Other" category includes Ephemeroptera, Mesostigmata, Plecoptera, Psocodea, Trichoptera and Trombidiformes.

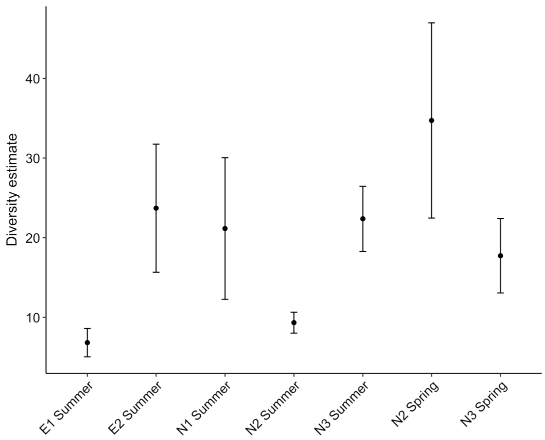

Figure 3 – Order 1 Hill diversity number estimates, and 95% confidence intervals at base coverage value of 0.550.

Hill number estimates show that colonies E1 and N3 had significantly lower richness values than colonies N1, N2 and E2 during the summer. On the other hand, while the richness value of the diet of N3 did not change significantly between seasons, N2 showed higher dietary diversity during the spring (Fig. 3).

Consumption of spiders

Frequency of occurrence of spiders changed between summer colonies (*p*=0.029) and between seasons (*p*=0.007). Adonis test also showed that the list of species changed significantly across colonies (F=4.785; d.f.=4; *p*=0.001) and seasons (F=4.169; d.f.=1; *p*=0.001). A total of 45 different species were recovered from the faecal samples. *Araneus diadematus* and *A. angulatus* during the summer (30 and 15 out of 92 bats), and *Nuctenea umbratica* during the spring (seven out of 14 bats), were the most frequent spider species.

We classified most of the spider species as vertical orb-web builders (21 out of 45) of the families Araneidae (16 species), Tetragnathidae (four species) and Uloboridae (one species). Other web builders were also identified, namely nine space-web builder species of the family Theridiidae, four funnel-web builders of the family Agelenidae, and one horizontal-web builder of the family Araneidae. Finally, some spiders that do not build webs were also detected, including ambushers/stalkers (five species), foliage runners (four species) and ground runners (one species) (Uetz et al., 1999; Roberts, 2001). Altogether, the species identified belonged to 12 families (Tab. 1).

The hunting strategy of the spiders occurring in the diet of *M. emarginatus* varied between colonies during the summer (F=3.982; d.f.=4; *p*=0.034): post-hoc comparisons grouped the composition of spider traits in colonies E1 and N1 from colonies N2 and N3 (for all significant pairwise tests: F>4.065; d.f.=1; *p*<0.035); any pairwise comparison involving E2 was not significant. Orb-web building spiders of the family Araneidae were the most common spider prey in all colonies (Fig. 4): their consumption was highest in colony E2, where all identified spiders belong to this group; followed by N1 and E1, in which orb-web building spiders reached 90.9% and 80% of occurrences respectively. In summer colonies N2 and N3, the incidence of orb-web building spiders was less prominent than elsewhere (POO<54%), as other spider guilds such as space-web builders were consumed in greater numbers (POO>15%); and in N2 funnel-web builders are 23% of the spiders consumed. Finally, non-web building spiders were also consumed, albeit occasionally, in every colony except E1 and E2 (Fig. 4).

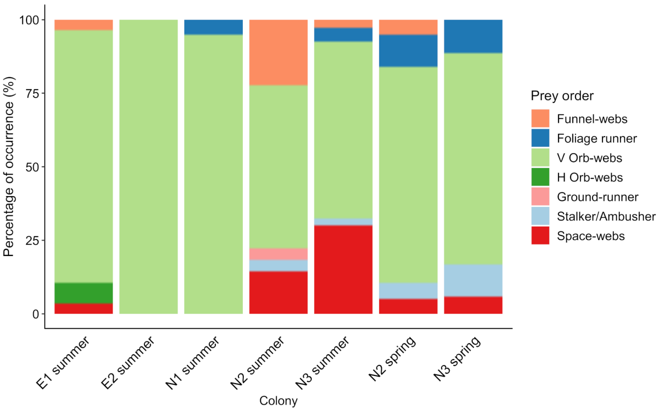

Figure 4 – Percent of occurrence (POO) of spider guilds identified in the diet from each colony. (H Orb-webs: Horizontal orb-webs; V Orb-webs: Vertical orb-webs).

Table 1 – Guild classification of identified spider species according to Uetz et al. (1999) and Roberts (2001).

Family	Species	Guild classification
Agelenidae	*Agelenatea redii*	Funnel-web builder
	Eratigena atrica	Funnel-web builder
	Tegenaria domestica	Funnel-web builder
	Tegenaria parietina	Funnel-web builder
Anyphaenidae	*Anyphaena accentuata*	Foliage runner
Araneidae	*Araneus angulatus*	Vertical Orb-web builder
	Araneus diadematus	Vertical Orb-web builder
	Araneus sturmi	Vertical Orb-web builder
	Aranues triguttatus	Vertical Orb-web builder
	Araniella cucurbitina	Vertical Orb-web builder
	Argiope bruennichi	Vertical Orb-web builder
	Argiope lobata	Vertical Orb-web builder
	Cyrtophora citricola	Horizontal web builder
	Gibbaranea gibbosa	Vertical Orb-web builder
	Larinioides cornutus	Vertical Orb-web builder
	Larinioides sclopetarius	Vertical Orb-web builder
	Mangora acalypha	Vertical Orb-web builder
	Neoscona subfusca	Vertical Orb-web builder
	Nuctenea umbratica	Vertical Orb-web builder
	Singa nitidula	Vertical Orb-web builder
	Zilla diodia	Vertical Orb-web builder
	Zygiella sp.	Vertical Orb-web builder
Clubionidae	*Clubiona brevipes*	Foliage runner
	Clubiona comta	Foliage runner
Eutichuridae	*Ceiracanthium elegans*	Foliage runner
Gnaphosidae	*Scotophaeus blackwallii*	Ground runner
Philodromidae	*Philodromus collinus*	Stalker/Ambusher
	Philodromus praedatus	Stalker/Ambusher
Tetragnathidae	*Tetragnatha extensa*	Vertical Orb-web builder
	Tetragnatha montana	Vertical Orb-web builder
	Tetragnatha nigrita	Vertical Orb-web builder
	Tetragnatha obtusa	Vertical Orb-web builder
Theridiidae	*Enoplognatha sp.*	Space-web builder
	Episinus maculipes	Space-web builder
	Parasteatoda tepidariorum	Space-web builder
	Platnickina tincta	Space-web builder
	Rhomphaea cf. rostrata	Space-web builder
	Rhompaea nasica	Space-web builder
	Steatoda grossa	Space-web builder
	Theridion melanurum	Space-web builder
	Theridion varians	Space-web builder
Thomsidae	*Xysticus lanio*	Ambusher
Uloboridae	*Hyptiotes flavidus*	Vertical Orb-web builder

We also found significant differences in the traits of spiders consumed in different seasons (F=3.072; d.f.=1; *p*=0.004), but none of the pairwise post-hoc tests were significant. Nonetheless, the consumption of orb-web building spiders did increase at the spring colonies, as all bats studied consumed them, as opposed to 48.3% of bats in colony N2, and 57.1% in colony N3 during the summer.

Discussion

Spiders were the most commonly consumed prey order, being identified in a total of 82 out of 106 bats' faeces. The frequency of occurrence of spiders in our localities was in the 62.1–100% range. Worldwide, spider remains have been found in 99% of the faeces of *K. papuensis* analysed in Australia (Schulz, 2000); while in the case of *M. keenii* in North America, spider consumption was recorded in 80% of the cases (Burles et al., 2008). Therefore, to our knowledge *M. emarginatus* is the third bat species of its kind described to date. Spiders have also been identified in the faeces of other bat species in North America (Whitaker et al., 1977; Whitaker, 2004; Kellner and Harestadi, 2005), and Europe (Ramos Pereira et al., 2002; Razgour et al., 2011; Hope et al., 2014); though their FOO does not exceed 25%.

Overall, the diet of *M. emarginatus* varied between locations. The differences of arthropod communities might promote the observed variations in the diet between colonies, mainly at the species level, a likely outcome given the generalist predatory character of bats and the intercolonial differences in landscape and climate (Clare et al., 2014a; Viglino et al., 2016; Aizpurua et al., 2018). Besides, while a few taxa — e.g.: *A. diadematus*, *A. angulatus* or *S. calcitrans* — were commonly consumed by many individuals across colonies, less frequent prey comprised most of the dietary list, with 55% of the species being consumed by a single bat individual (Tab. S2). Thus, differences in the dietary composition were amplified when the prey species level was considered.

Colonies N1, E1 and E2 show similar diet at the ordinal level, as spiders and lepidopterans were the main prey. Goiti et al. (2011) described a comparable diet in Central Iberian Peninsula and proposed it as the foraging archetype closest to the bat's original situation. In both studies, the colonies studied were in areas of warmer, dryer climate of Mediterranean characteristics. Even though *M. emarginatus* is a bat mostly found along the Mediterranean (Piraccini, 2016), the majority of studies on its foraging ecology have been conducted in its Northernmost distribution range, where it often adopts an opportunistic foraging behaviour (Beck, 1995; Zahn et al., 2010; Kervyn et al., 2012; Dekker et al., 2013). This calls attention to the fact that our current view of *M. emarginatus*'s trophic niche could be highly biased, and further studies across its entire distribution range would help to describe its typical foraging behaviour and preferred prey types.

Despite observed specialization on spiders in the three Mediterranean colonies, summer results in colonies N2 and N3 suggest that *M. emarginatus* shows a certain degree of flexibility and adaptability, which allows it to opportunistically hunt punctually available prey and/or exploit different foraging grounds. Thanks to this opportunist facet, *M. emarginatus* would not be as susceptible to shifts in prey abundance as other strictly specialist predators would be (Maine and Boyles, 2015), which would allow it to successfully adapt to modified environments and anthropogenic landscapes.

We found that the diet composition — and richness in the case of colony N2 — changed significantly between seasons in colonies N2 and N3. Overall, from spring to summer, consumption of spiders decreased, while that of dipterans increased. On top of that, in colony N3, half the bats studied consumed *A. rusticus* during summer, but not during the spring. Its relatively high local frequency was probably linked to a seasonal increase in its density in conifer plantations surrounding the colony. Flaquer et al. (2008) also reported that *M. emarginatus* foraged in pinewood plantations in Mediterranean Iberian Peninsula, even though it seems to avoid such foraging grounds in Central Europe in favour of native, deciduous woodlands (Zahn et al., 2010; Dekker et al., 2013).

Summer diet of colony N2, and partly in N3, resemble those described by Beck (1995) and Kervyn et al. (2012): the primary food source are dipterans, followed by spiders. The abundance of flies such as *Musca sp.* and *S. calcitrans* in the diet of *M. emarginatus* has been linked to the use of cattle sheds as foraging grounds, as reported in colonies of Central Europe (Kervyn et al., 2012; Dekker et al., 2013). Cattle are abundant in colony N2 (108 cows/km^2, www.bizkaia.eus), and open grasslands and pastures mostly cover the surrounding area. This hypothesis is supported by the fact that traces of bovine DNA were identified in the faeces of 30 out of the 47 bats that consumed *S. calcitrans* in colonies N2 and N3. In this case, the detection of cow DNA would be a result of secondary predation of flies on cow blood (Sheppard et al., 2005; Patra et al., 2018).

The presence of *S. calcitrans* around livestock can cause several negative effects on the cattle, causing reduction of weight and/or milk production, and acting as a vector of disease (reviewed in Patra et al., 2018). In our study, *S. calcitrans* was the most frequently consumed prey species, as it was identified in 58% of bats in total, and in 82% of bats specifically in the summer colonies N2 and N3. Due to the huge number of insects consumed by an individual per night and their generalist nature, bats have often been mentioned as potential top-down suppressor of agricultural insect pests (Kunz et al., 2011). The high FOO values of *S. calcitrans* suggest that *M. emarginatus* could act as a potential suppressor of cattle flies, as well as other insects which, in high densities, cause harm to cattle.

Cow sheds can be a convenient source of prey for many bat species throughout the year (Dekker et al., 2013); they provide a more constant source of insects for bats (Zahn et al., 2010), as they are less affected by local weather and temperature drops. Nevertheless, the higher occurrence of stable flies recorded during the summer does not seem to support this hypothesis; instead, an increase in the densities of flying insects — including cattle related flies like *S. calcitrans*, *Musca autumnalis* or *Muscina stabulans*— linked to warmer summer temperatures (LaBrecque et al, 1972) is a more likely explanation.

Regarding spiders, most species consumed belong to the vertical orb-web building guild, which were especially prominent in colonies N1, E1 and E2. These have also been proposed as the main guild consumed by other spider specialist bats (Schulz, 2000; Burles et al., 2008). *M. emarginatus* most likely hunts them directly from their webs, and could also feed on insects trapped on them. However, the exact mechanism used to detect and catch spiders while in their webs is not clear, as both gleaning (Schulz, 2000; Kervyn et al., 2012) and hawking (Goiti et al., 2011) have been proposed as possible strategies. The consumption of other types of prey suggests that *M. emarginatus* does have the ability to glean. Diurnal flies such as *Musca sp.* or *S. calcitrans* could be hunted from cattle-shed walls by gleaning (Krull et al., 1991), and/or stolen from orb-webs where they got trapped; and non-web building spiders such as *Anyphaena accentuata*, *Clubiona comta* or *Xysticus lanio* were likely caught from the surface of the vegetation.

The overall abundance of orb- and space-web building spiders in all colonies indicates that environments of cluttered vegetation, e.g. inside forests, are the most likely foraging grounds of *M. emarginatus* (Dekker et al., 2013; Flaquer et al., 2008; Zahn et al., 2010). Abundance and diversity of aerial-web building spiders is higher in such areas, as they provide plenty of anchoring points to build their webs (Balfour and Rypstra, 1998). Funnel-web builders, on the other hand, build their webs closer to the ground, and are commonly found inside buildings (Roberts, 2001); therefore, their consumption may be linked to the use of such foraging grounds, especially in colony N2.

Gleaning and foraging in cluttered environments are shared characteristics between the other spider specialist bats, and are thought to be important in the development of such dietary specialization (Schulz, 2000; Burles et al., 2008). However, they are not mutually exclusive, as they are also found amongst other bat species. for example, *P. auritus*, *Myotis bechsteinii*, *M. myotis* or *M. nattereri*, forest species which are known to glean over prey (Norberg and Rayner, 1987; Anderson and Racey, 1991; Arlettaz, 1996; Napal et al., 2013; Swift and Racey, 2002), although they do not depend on spiders as extensively as *M. emarginatus* (Beck, 1995; Ramos Pereira et al., 2002; Razgour et al., 2011; Hope et al., 2014).

Spiders, being predators, are less abundant than other arthropod taxa (Pimm, 1988), which may explain why very few bat species consume them in great quantities. While such dietary specialization may allow spider eating bats to reduce competition, it is also advantageous when the abundance of other resources, mainly flying insects, is lower due to adverse weather conditions (Burles et al., 2009), or during seasons of lower insect abundance. This emphasizes the uniqueness of *M. emarginatus* and other spider specialist bats regarding dietary choices, and hints at specific adaptations regarding sensorial ecology and behaviour.

Conclusions

Using molecular techniques, we studied the diet of five colonies of *M. emarginatus* at two different seasons, and we were able to identify a total of 138 prey species, 45 of which were spiders. These results assure that these preys play an important role in the diet of *M. emarginatus*, and stress its uniqueness amongst European bats. In fact, *M. emarginatus* seems to focus a great part of its diet on spiders, principally those belonging to the vertical orb-web building guild, which are presumably caught directly from their webs in cluttered environments. The exact mechanisms involved in the detection and obtaining of such prey are not yet understood. This knowledge could potentially provide insights into why they are so available for *M. emarginatus*, while rarely recorded in the diet of other European forest species. On the other hand, colonies N2 and N3 also showed that *M. emarginatus* has a certain degree of geographical and seasonal variability, and can opportunistically exploit different resources, which emphasizes the fact that both landscape use and overall resource availability ultimately shape the foraging niche of a bat population.

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Supplemental information

Additional Supplemental Information may be found in the online version of this article:

Supplemental Table S1 Complete list of identified OTUs.

Supplemental Table S2 Complete list of taxa identified, with absolute frequency of occurrence in each colony and season.