



# Seasonal shift in the diet of the notched-eared bat (*Myotis emarginatus*) in the Basque Country: from flies to spiders

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## Abstract

*Myotis emarginatus* seems fond of spiders and flies, a unique feeding style among European bats. The importance of each prey type varies among studies, so this paper aims to expand on the knowledge to unveil the trophic niche of *M. emarginatus* by studying its diet changes throughout the maternity season. We sampled five maternity colonies in the Basque Country every 2 weeks for the whole duration of the maternity season and studied their diet using DNA metabarcoding methods. We observed significant changes in diet diversity and composition. At the beginning of the season, *M. emarginatus* consumed a variety of prey orders, Diptera being the most abundant; but as the season progressed, the relative consumption of Araneae increased. By August, 80% of the weighted percentage of occurrences (wPOO) corresponded to the orb-web-building spider *Araneus diadematus*. Orb-web-building spiders need habitats of high vertical complexity to grow in abundance. Therefore, conserving them is essential to preserve attractive hunting grounds for colonies of *M. emarginatus*. Besides, diurnal flies, mainly *Stomoxys calcitrans*, were also considerably consumed in the colonies studied, which can become detrimental to the well-being of cattle, and can act as a vector of many diseases.

**Keywords** *Myotis emarginatus* · Seasonal diet · Prey · *Stomoxys calcitrans* · *Araneus* · DNA metabarcoding

## Introduction

Since the beginning of the twenty-first century, the diet of insectivorous bats has been widely studied, especially in Holarctic regions (Taweesub et al. 2021). This is undoubtedly linked to the rise in popularity of DNA-Metabarcoding and High Throughput Sequencing techniques, which offer an easy and cost-effective way to comprehensively analyse the diet of bat individuals and colonies (Pompanon et al. 2012). Although limitations of molecular techniques are still under discussion (Alberdi et al. 2018; Littleford-Colquhoun et al. 2022), they have provided valuable insight into hidden aspects of the foraging ecology of bats (Andriollo et al. 2021; Arrizabalaga-Escudero et al. 2019) and the ecosystem

services they provide (Baroja et al. 2019; Garin et al. 2019; Taylor et al. 2018). The basic knowledge elicited by dietary studies has also paved the way to understand ecological processes beyond mere prey consumption (Alberdi et al. 2012; Arrizabalaga-Escudero et al. 2015) serving as a valuable tool for the successful management of human and natural resources (Cuff et al. 2022).

Both molecular and previous morphologic studies have found that the dietary range of bats can be highly diverse and variable. Each species is constrained by morpho-ecological characteristics (Emrich et al. 2014), which limit habitat use (Starik et al. 2021) and prey detectability (Siemers and Swift 2006). Nevertheless, the diet of each bat species has been reported to vary according to intrinsic characteristics of individuals like sex (Mata et al. 2016) or age (Arrizabalaga-Escudero et al. 2019), as well as factors affecting prey availability, such as seasonality, weather, landscape, or the presence of competing species (e.g. Goiti et al. 2008; Napal et al. 2013; Novella-Fernandez et al. 2020; Razgour et al. 2011; Tournayre et al. 2020a; but see Wray et al. 2021).

This paper aims to provide insight into the diet of the Notch-Eared bat (*Myotis emarginatus*) in a temperate-oceanic region throughout the whole duration of the maternity

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season. Previous studies on the diet of *M. emarginatus* have shown unique characteristics among European bat species because of the high prevalence of orb-weaving spiders (Araneidae) and diurnal flies (Muscidae) (Bauerova 1986; Beck 1995; Goiti et al. 2011; Kervyn et al. 2012; Krull et al. 1991; Vallejo et al. 2019). While spiders are common prey for bats of the gleaning guild (e.g. Andreas et al. 2012a; Roswag et al. 2018), only three species have been described as spider specialists among chiropterans: *Kerivoula papuensis* (Schulz 2000), *Myotis evotis* (Burles et al. 2008) and *Myotis emarginatus* (Goiti et al. 2011); but see Novella-Fernandez et al. (2020). Spiders are the primary food source for these bat species, who consume them consistently. Molecular analyses have identified 45 different spider species in the *M. emarginatus* faeces in the Iberian Peninsula, and vertical orb-web weaving spiders of the family Araneidae are the most common spider prey item in all the studied locations (Vallejo et al. 2019). On the other hand, the consumption of the blood-sucking diurnal cattle-fly *Stomoxys calcitrans* is especially noteworthy. This fondness for flies has been reported to account for over half of *M. emarginatus*' diet in Central Europe (Beck 1995; Kervyn et al. 2012; Steck and Brinkmann 2006) and some colonies in the Iberian Peninsula (Vallejo et al. 2019). It seems to be linked to the presence of cattle sheds in the foraging grounds, where *M. emarginatus* has commonly been reported to forage (Dekker et al. 2013; Dietz and Pir 2021; Krull et al. 1991).

Generalist predators, like insectivorous bats, shift from selective to opportunistic behaviour depending on the abundance of their preferred prey (Emlen 1966). For example, the Mediterranean horseshoe bat (*Rhinolophus euryale*) consumes a diverse diet in spring. However, it shifts to feed almost exclusively on moths during the summer, when flying insects reach their yearly peak (Hails 1982). This prey item fits better into the detection and hunting features of the bat (Goiti et al. 2008). Similarly, *Myotis myotis* consumes more ground dwelling spiders during the summer, when the overall abundance of its preferred prey, carabids and crickets, drops (Ramos-Pereira et al. 2002).

Kervyn et al. (2012) found a higher proportion of moths in the diet of *Myotis emarginatus* studied in May than in the rest of the breeding season but found no significant changes in diet composition otherwise. Vallejo et al. (2019) suggested the importance of spiders decreases from spring to summer, in favour of cattle flies and other occasional prey. Nonetheless, the understanding of how the diet of *M. emarginatus* can change seasonally is still scarce.

In this research, we aim to describe temporal changes in the diet of *M. emarginatus* in five maternity colonies in the Basque Country (Northern Iberian Peninsula). To do so, we investigated the changes in diet composition and diversity of this bat species every fortnight in maternity colonies. The diet of the notch-eared bat has previously been studied in the

region by Vallejo et al. (2019) and Goiti (unpublished data) and found that both spiders and stable flies were abundant, the latter, especially during the summer months. Based on these results, we expected the diet composition and diversity to vary seasonally and precisely predict that (1) the diet would be more diverse in spring than in summer, (2) the proportion of spiders consumed will be high overall, and (3) the consumption of diurnal flies would be highest during the summer months. A decrease in the dietary diversity during the summer months, and an increase in the proportion of cattle flies consumed would indicate that cattle flies are an essential food source for *M. emarginatus* in this area, as it focuses on them in times of higher overall resource abundance, while it maintains a more diverse diet in times of lower resource abundance (Emlen 1966).

## Materials and methods

### Sample collection

The five maternity colonies studied are within a 27 km radius (the Basque Country, Southwestern Europe) and all are roosting in artificial constructions. The climate in the region is temperate oceanic, with mild temperatures throughout the year (mean: 14°C) and abundant precipitation (1200–2000 mm). The landscape around all colonies is highly modified by human activity, as pine and eucalyptus plantations are common in the region.

#### Baranbio (BA)

A farm house, where around 100 *M. emarginatus* share the roost with 40 *Rhinolophus ferrumequinum*. The colony is surrounded by coniferous and eucalyptus plantations on a 5 km radius, but further away (up to 10 km) broad-leaved forests, pastures, herbaceous crops and scrubland are available.

#### Ereño (ER)

An empty building in an abandoned quarry gives roost to up to 50 *M. emarginatus*. Around 15 *R. ferrumequinum* and occasionally some *Rhinolophus euryale* are also present. It is surrounded by a diverse landscape composed of broad-leaved forests (including evergreen oak forests), pine and eucalyptus plantations, and wetland.

#### Lauro (LA)

Around 20 *M. emarginatus* roost in an abandoned unfinished two-storey construction. The area is surrounded by

agricultural land of various types and a few patches of broad-leaved forests, eucalyptus plantations, pastures and scrublands.

### **Mañaria (MA)**

A church vault where up to 200 *M. emarginatus* share the space with *R. ferrumequinum*. Mostly broad-leaved forests and pine plantations surround the area.

### **Zestoa (ZE)**

The biggest colony of *M. emarginatus* in the region, with more than 500 individuals, sharing the roost with around 100 *R. ferrumequinum* and 30 *Miniopterus schreibersii* in an empty building, right next to a river bed. Pastures, meadows, broad-leaved forests and a few pine plantations surround the area.

We sampled these five colonies in the year 2020. Bats at ZE arrived the earliest (May 4th, Week 19), and left the latest (September 3rd, Week 36). On the other hand, bats from LA spent the shortest time in the roost, arriving the latest (May 29th, Week 22) and leaving the earliest (August 5th, Week 32).

We took special care to reduce disturbance to the colonies. Every fortnight, a collector was placed under the bats and faeces were collected after no more than 2 days to minimize DNA degradation. We collected up to 20 samples of 4–6 pellets each. In the cases where the risk of contamination from the faeces of non-target bat species was high, we collected up to 40 samples containing one or two pellets each. Sampling was always conducted in even weeks. Exceptionally, four collections were taken in the next week, although they were analysed as if they were collected in the week before: ZE in week 18, ER in week 20, LA in week 28 and BA in week 30.

Samples from each colony and sampling date were processed and sequenced to describe 75% of the diet diversity per colony and day (Chao et al. 2014). Unfortunately, we could only reach such sequencing depth in some of the colonies and dates due to budgetary reasons. We performed inter- and extrapolation of dietary richness using package iNEXT (Hsieh et al. 2020). In total, 595 samples were sequenced. One extraction blank was added every 23 samples, and one library blank was included in every MiSeq Run performed.

### **DNA extraction, PCR amplification and sequencing**

The faeces were weighted, and the DNA was extracted using DNeasy PowerSoil Kit and DNeasy PowerSoil Pro Kit (Qiagen) following manufacturer's instructions with some modifications. For the amplification process, we used primer set FWH1 (Vamos et al. 2017), which targets a 180 bp region

of the COI gene. This primer set was chosen because: first, it amplifies a broad range of prey taxa, and also amplifies bat DNA (Tournayre et al. 2020b) which is helpful to detect contamination from non-target bat species in our samples; and second, it is longer than other popular primer sets used in bat dietary studies (e.g. Gillet et al. 2015; Zeale et al. 2011), which reduces the chance to amplify DNA from unwanted sources. Shorter markers can capture greater diversity (Elbrecht et al. 2019; Tournayre et al. 2020b) but are also prone to amplify very degraded and unwanted DNA coming, for example, from secondary predation (Galan et al. 2018). Usually this is not concerning, but given the fact that spiders are expected to be abundant in the diet of *M. emarginatus*, the probability of detecting DNA from secondary predation increases, and it would hamper its discrimination from positive data.

PCR amplification was performed following Tournayre et al. (2020b), with modifications. Libraries were built using Illumina's Nextera XT kit, and samples were sequenced in Illumina MiSeq. PCR amplification, DNA library construction and sequencing processes were done at the Genomics and Proteomics General Service (SGIker) of the University of the Basque Country (UPV/EHU).

Details on all laboratory procedures can be found in Online Resource 1.

### **Sequence analysis and taxonomic assignment**

Bioinformatic analyses were done using vsearch (Rognes et al. 2016) and Cutadapt (Martin 2011), following the steps described in Esnaola et al. (2018) with the necessary modifications. Details are explained in Online Resource 1.

The 595 samples (Online Resource 2) analysed yielded 7851 OTUs (Online Resource 3) which were assigned to their appropriate taxonomy using the *blastn* function in BLAST+ (Camacho et al. 2009) to access the GenBank dataset, and Boldigger-cline (Buchner and Leese 2020) to access the BOLD dataset. Only matches over 98% similarity were considered. The output of both datasets was curated manually and using a custom script so that each OTU was only assigned to a single taxon. Each assigned taxon was classified into one of the following categories: predator, prey, not-in-study-area, environmental contamination and unassigned.

### **Selection of samples and OTUs**

Samples were checked for contamination from potential prey species by studying the sample blanks. We found no clear contamination event from species that could be mistaken for prey. Nonetheless, an abundance threshold was applied to remove taxa with low read abundances, so that OTUs with less than 0.5% of reads were removed in each sample.

While far from perfect, this method has proven to be quite effective in limiting contamination risk of multiple sources without eliminating too many rare prey taxa (Drake et al. 2022); therefore, the data were interpreted having this decision in mind.

We also checked for contamination from co-occurring bat species. Samples were discarded from the analysis if more than 10% of all the reads identified as bat species belonged to others than *M. emarginatus*. We obtained at least 8–12 samples eligible for analysis in most situations. Nevertheless, we removed samples from MA in weeks 20, 32 and 34, and of ZE in week 36 groups entirely from the analysis, because we did not manage to obtain more than one viable sample for the analyses. More information on the selection of samples is given in Online Resource 1.

### Description of dietary metrics

All data analysis was performed in R version 4.0.4 (R Core Team 2021). Only those OTUs classified as potential prey were used for the diet analysis. Due to the lack of proven correlation between relative read abundances (RRA) and biomass of prey consumed (Paula et al. 2022), we converted our data to weighted percentages of occurrences (wPOO), a metric based on presence/absence data that provides a good proxy of consumption (Cuff et al. 2022; Deagle et al. 2018). We calculated wPOO values at the prey species, genus, family and order levels.

### Multivariate analysis of diet variability and homogeneity

We explored diet composition and variability at the species, genus, family and order level of prey items. We calculated Bray–Curtis dissimilarities between all samples using function *vegdist* in package *vegan* (Oksanen et al. 2020). We explored relationships between diet composition and Week, Colony and their interaction through a Permutational Multivariate Analysis of Variance, or PERMANOVA (Anderson 2001), using function *adonis2* in package *vegan*.

We calculated distances of samples to the centroid of their respective group in non-Euclidean space using function *betadisper* in package *vegan* to test for homogeneity of multivariate dispersion (Anderson and Walsh 2013). We performed the analysis by defining groups of samples by the combination of Week and Colony. We used the results of these analyses to explore changes in diet variability, that is, beta diversity (Anderson et al. 2006). To avoid the creation of negative eigenvalues in the process, we calculated square root transformed distances, instead of standard ones.

The distances calculated by *betadisper* were extracted and modelled against factors Week, Colony and their interaction in a conventional linear regression, to explore differences in

diet variability. We visualized the marginal effects of each factor in the model and their interaction using package *margins* (Leeper 2021). In addition, we represented each group's centroid position calculated by *betadisper* in a PCoA to analyse underlying patterns in the overall composition of the sample groups and their relationship with each other.

### Analysis of seasonal diet composition

Finally, the effect of sampling date on the diet composition was analysed at the ordinal level using a Multinomial Logit model, which is used to model discrete choices between mutually exclusive alternatives (Croissant 2017). They have been recently used to model changes in diet composition in bats (Tiede et al. 2020) and they can theoretically define the probability that an alternative—a prey item in our case—is chosen (see Croissant 2017). To run the model, we collapsed all orders found in less than 15 samples in a new category called “Others”, resulting in six different prey orders.

We chose to use package *brms* (Bürkner 2017), which uses Markov chain Monte Carlo (MCMC) algorithms to fit various multilevel models, including multinomial logit models, under a Bayesian framework. We chose the category “Others” as a baseline for the model. We calculated the estimated probabilities of consumption for each order every week and the marginal effect of the week on the consumption of each order, without the effect of the baseline category.

## Results

### Selection of samples and OTUs

After removing non-eligible samples (Online Resource 2), 359 samples were left for the diet analysis, which yielded a total of 6314 OTUs, of which only 664 had an abundance higher than 0.5% in any of the samples (Online Resource 3). Seven OTUs (accounting for 50% of total reads) belonged to the predator, *M. emarginatus*; 73 (4% of reads) were classified as environmental contamination; 251 (24% of reads) were identified as potential prey items; four belonged to potential prey species that are not found in the study area (0.5% of reads); and the remaining 329 (21% of reads) did not match with any sequence in the databases at least at the 98% identity level. In total, 155 unique prey species and 21 unique genera were identified. In addition, seven genera also had OTUs identified both at the genus and the species level. In these cases, a genus level occurrence was added to the dietary data. All in all, we analysed 183 prey taxa, identified at the genus or species levels, belonging to 92 families and 14 orders.

The most frequent prey species was the orb-weaving spider *Araneus diadematus*. It was identified in 249 samples (Frequency of occurrence (FOO) 69%) and accounted for 42% RRA and 33.9% wPOO. The next two most frequent prey items were *Stomoxys calcitrans* and *Musca domestica*, identified in 81 and 68 samples, with 9.8% and 6.6% RRA and 7.8% and 6.7% wPOO, respectively. Most prey items appeared occasionally in faeces: 108 prey species were only identified in a single sample each, 22 were identified in two samples, and 37 in between three and ten samples. Therefore, only 16 species occurred in more than ten samples out of 359 (Table 1). Half of them were orb-web weaving spiders.

Araneae and Diptera were the two most consumed prey orders, accounting for 67% (Weeks 18–20) to 88% (Weeks 34–36) of the total wPOO. During May, the consumption of other orders is noteworthy: *Caenis luctuosa* (Ephemeroptera) had the highest wPOO (37%) in ZE Week 18; *Pharmacis fusconebulosa* (Lepidoptera) was also the highest consumed prey item (wPOO: 27%) in BA Week 20; *Melolontha melolontha* (Coleoptera) was the third most consumed prey item (wPOO 13%) in ER Week 20.

### Multivariate analysis of diet composition and variability

PERMANOVA showed significant difference in diet composition across Week, Colony and the interaction of both factors at all taxonomical levels ( $\alpha=0.05$ ). Figure 1 shows a PCoA representing the centroid position of every group of samples in a non-Euclidean space, as calculated by *betadis-per*. The first two PCoA axes represent 53% of the total variability at the order level, but a lower percentage at other

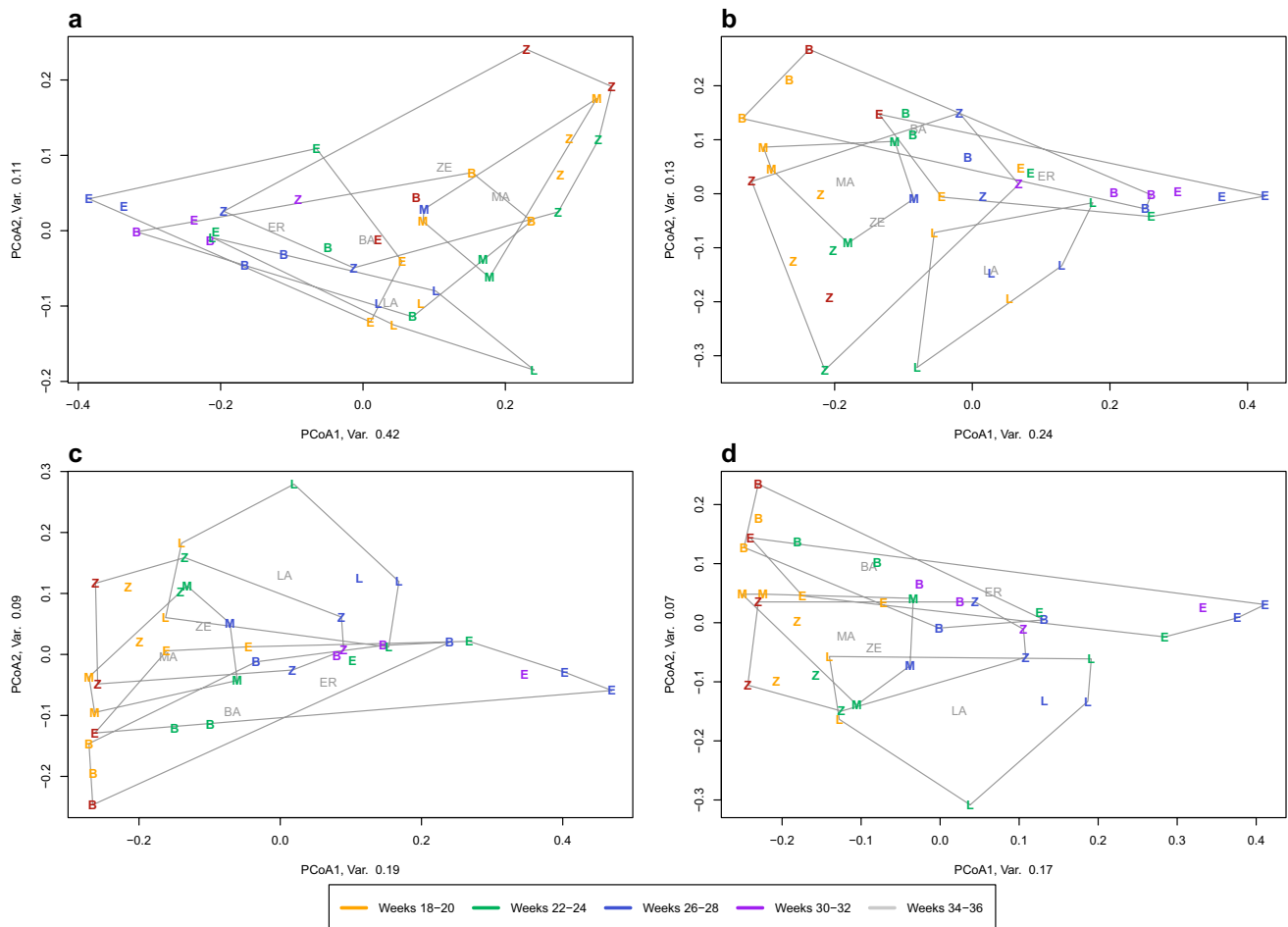
taxonomic levels. The first axis separates groups of samples similarly regardless of the taxonomic level: it separates samples from the first four sampling weeks (18–24) and the last four sampling weeks (30–36). Mid-season samples appear all along the first PCoA axis, closer to early or late season samples depending on Colony (Fig. 1). The second PCoA axis separates certain colonies more and shows different patterns depending on the taxonomical rank used. *betadis-per* also showed significant differences in the diet variability of Colonies, Week, and a combination of both factors at all taxonomical levels ( $\alpha=0.05$ ). The distances to centroid calculated by *betadis-per* were negatively correlated to Week at all taxonomical levels (Order:  $F_1=62.161$ ,  $p<0.001$ —Family:  $F_1=86.5237$ ,  $p<0.001$ —Genus:  $F_1=90.024$ ,  $p<0.001$ —Species:  $F_1=71.262$ ,  $p<0.001$ , Fig. 2a), meaning that diet variability decreased with Week. Distances to centroid were also significantly different between colonies at all taxonomical levels (Order:  $F_4=14.864$ ,  $p<0.001$ —Family:  $F_4=25.277$ ,  $p<0.001$ —Genus:  $F_4=31.367$ ,  $p<0.001$ —Species:  $F_4=31.000$ ,  $p<0.001$ ). Colonies LA and ER had significantly lower average distances to the centroid (Fig. 2b). An interaction between Week and Colony showed that the temporal pattern might vary in different ways across Colonies (Order:  $F_4=5.647$ ,  $p<0.001$ —Family:  $F_4=7.429$ ,  $p<0.001$ —Genus:  $F_4=11.335$ ,  $p<0.001$ —Species:  $F_4=6.967$ ,  $p<0.001$ ). ER and BA presented a sharper decrease in diet variability as the season progressed (Fig. 2c, d), compared to the rest of the colonies (Fig. 2e–g).

### Seasonal changes in diet composition

Given that most variability happened through time (Fig. 1), we modelled the changes in the proportion of six prey orders

**Table 1** Prey species that were identified in more than ten samples, and their global frequency of occurrence (FOO) and weighted percentage of occurrence (wPOO)

Order	Family	Species	FOO	wPOO
Araneae	Araneidae	<i>Araneus diadematus</i>	249	33.87
Diptera	Muscidae	<i>Stomoxys calcitrans</i>	81	7.85
Diptera	Muscidae	<i>Musca domestica</i>	68	6.78
Araneae	Araneidae	<i>Nuctenea umbratica</i>	67	5.20
Araneae	Tetragnathidae	<i>Metellina merianae</i>	58	4.66
Araneae	Tetragnathidae	<i>Metellina sp.</i>	46	3.42
Diptera	Limoniidae	<i>Limonia nubeculosa</i>	28	1.82
Araneae	Araneidae	<i>Zygiella sp.</i>	25	1.91
Ephemeroptera	Caenidae	<i>Caenis luctuosa</i>	22	2.91
Araneae	Araneidae	<i>Cyclosa conica</i>	21	1.32
Araneae	Araneidae	<i>Araneus angulatus</i>	20	1.67
Lepidoptera	Hepialidae	<i>Pharmacis fusconebulosa</i>	18	1.87
Diptera	Tipulidae	<i>Tipula fulvipennis</i>	16	0.98
Araneae	Tetragnathidae	<i>Tetragnatha sp.</i>	16	1.49
Araneae	Uloboridae	<i>Hyptiotes paradoxus</i>	15	0.95
Araneae	Araneidae	<i>Araneus triguttatus</i>	14	0.78



**Fig. 1** PCoA ordination of the position of the centroids of groups of samples collected in the same day and Colony, with prey items classified to the **a** Order level, **b** Family level, **c** Genus level and **d** Species

level. The proportion of the variability explained by each of the axes is indicated in the axis labels

simultaneously in relation to Week using Multinomial logit models (Fig. 3). The proportion of Araneae increased significantly with Week, compared to the baseline (95% CI 9–12%). The frequency of Araneae was estimated to increase by 3.1% every week (95% CI 2.9–3.1%). On the other hand, Diptera, Lepidoptera and Ephemeroptera, and the baseline Others decreased significantly, albeit the estimated decrease for Diptera is the highest, at 1.9% points every week (95% CI Diptera – 2 to – 1.8%, Lepidoptera – 0.7 to – 0.6%, Ephemeroptera – 0.3 to – 0.2%, Others – 0.13 to – 0.06%).

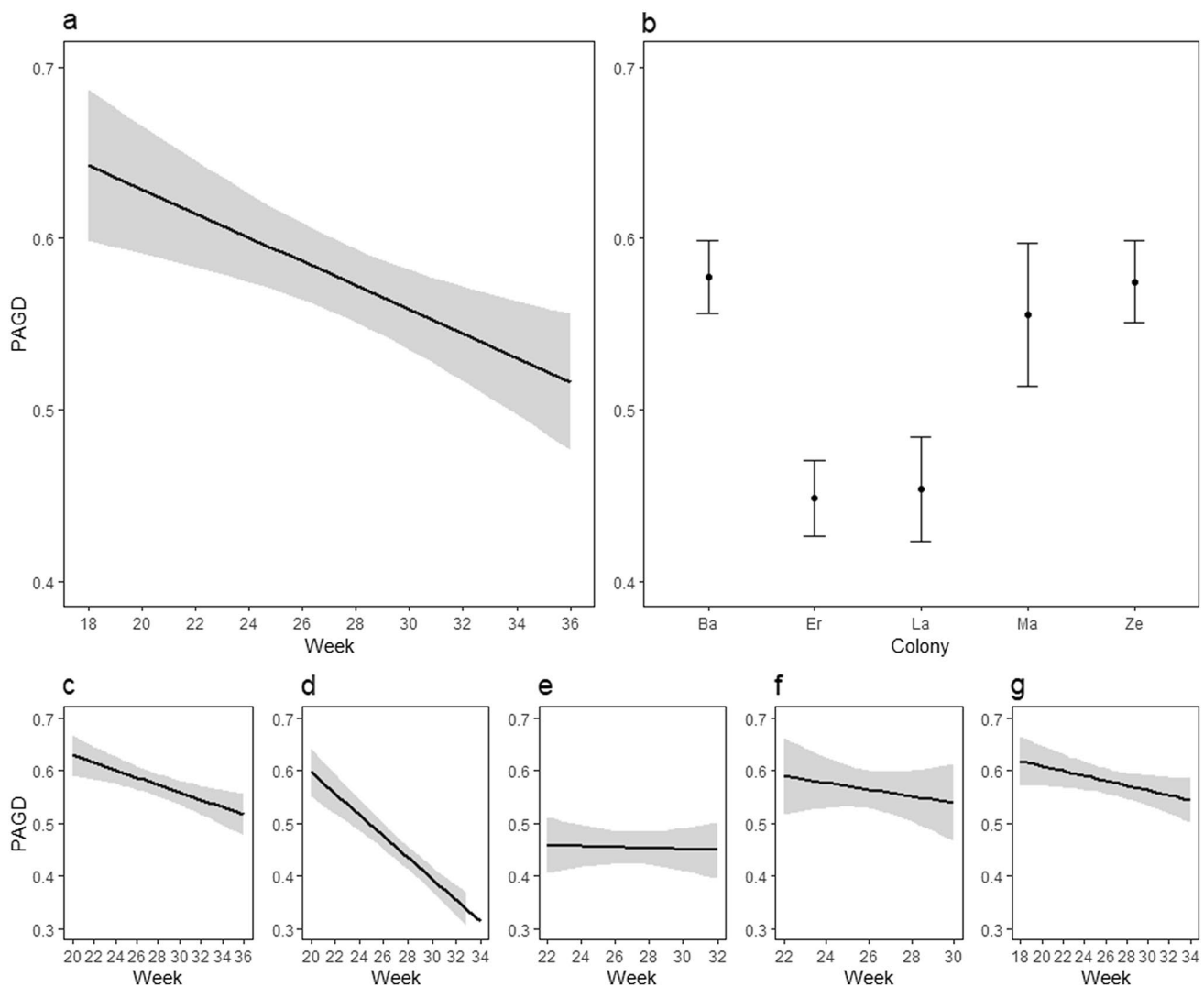
Overall, the model estimated that the consumption of Araneae would increase from a wPOO value of 28% (95% CI 10–49%) at Week 18, to a wPOO of 84% at Week 36 (95% CI 63–93%). Similarly, Diptera decreased from 46% (95% CI 19–74%) to 9% (95% CI 2.6%–27%) (Fig. 3). The confidence intervals of all orders overlapped at the beginning of the season. While Diptera showed the highest wPOO then, we cannot say that it was consumed significantly more than any other order. That situation quickly

changed, as the consumption of Araneae became increasingly common. It reached up to 80% wPOO at the end of the season. As its confidence intervals did not overlap with the rest of the orders, Araneae was consumed significantly more than the rest of the orders at the end of the summer.

## Discussion

### Seasonal changes in diet variability and composition

Our results showed that both the variability and the composition of the diet of *Myotis emarginatus* changed throughout the season in the five colonies studied at multiple taxonomical levels. At the beginning of the season, Diptera was the most consumed prey order. However, they were not consumed significantly more than any other order (Fig. 3). As the season progressed, the relative importance of Araneae



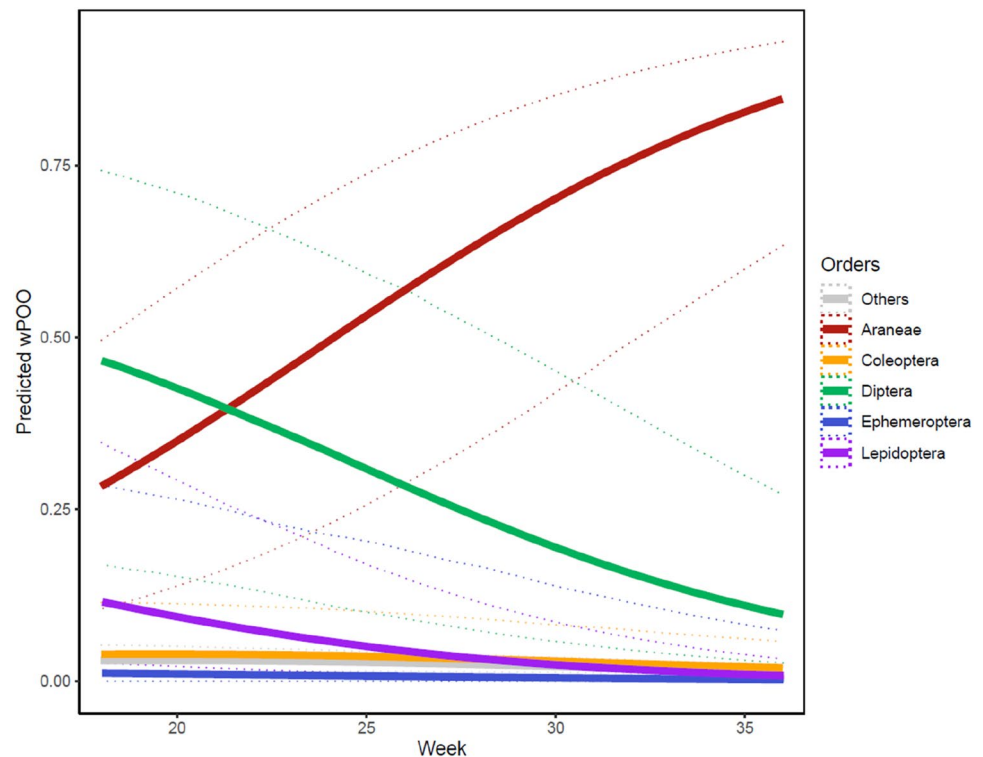
**Fig. 2** Marginal effects of Predicted Average Distance to Centroid (PADG) and 95% confidence intervals calculated by the linear model of distances to group centroids (at prey species level) against Week, Colony and their interaction. Plot **a** shows the marginal effect of

Week PADG without the effect of Colony, plot **b** shows PADG per colony, without the effect of seasonality, and plots **c–g** show the colony PADG: **c** BA, **d** ER, **e** LA, **f** MA, **g** ZE. The effects for the models at different taxonomical levels are similar

in the diet gradually increased, and by the end of the season, spiders, particularly *Araneus diadematus*, constituted the main prey item of *Myotis emarginatus* in the colonies studied. This suggests that orb-web building spiders are the preferred prey for *Myotis emarginatus* in this area. Additionally, some prey items were also consumed only locally, and in abundance, during the first weeks of the bat's stay in the breeding roosts, like *C. luctuosa* (Ephemeroptera) in ZE, *P. fusconebulosa* (Lepidoptera) in BA or *M. melolontha* (Coleoptera) in ER. Kervyn et al. (2012) also reported higher proportions of Lepidoptera in the diet of *M. emarginatus* at the beginning of the season. Colony ZE is located next to a river, which might explain the occasional high consumption of *C. luctuosa*.

As we predicted, the diet of *M. emarginatus* was more diverse at the beginning of the season, which may be a response to overall lower availability of prey, as both flying insects and spiders show a peak of abundance in late summer (Hails 1982; Hsieh and Linsenmair 2012). In the study region, the abundance of the stable fly *Stomoxys calcitrans* peaks during late summer-early autumn (Valbuena-Lacarra and Saloña-Bordas 2010). The consumption of a more diverse diet at times of a priori lower resource availability has also been reported in other bat species (Andreas et al. 2012b; Presetnik and Aulagnier 2013, see Introduction). However, this is not the norm, as some studies show that bats opportunistically feed on available resources, and thus the diversity of their diet mirrors that of available prey (Divoll et al. 2022; Tiede et al. 2020).

**Fig. 3** Estimated probabilities of weighted percentage of occurrence (wPOO) values of prey orders estimated by the multinomial logit model, with 95% confidence intervals



Being highly mobile, bats can respond quickly to changes in resource availability, such as those created by pest species of patchy distribution and short flying stages (Aizpurua et al. 2018; Baroja et al. 2021). This behaviour underlines insectivorous bats' plasticity and capacity to exploit various resources (Faure and Barklay 1994; Tournayre et al. 2020a).

Diet diversity did not change equally in all colonies studied; it decreased noticeably (Fig. 2c, d) in BA and ER, but not in the rest. Since the time series for colonies MA and LA were shorter, we might have been prevented from observing a significant decrease in diversity in these colonies too. Additionally, local differences in the arthropod communities could result in the observed differences regarding diet variability and composition. Finally, we did not measure local arthropod abundance and diversity in this study, which would be essential to fully understand whether and how *M. emarginatus*, and other insectivorous bats, adjust their foraging ecology and responds to changes in prey availability.

### Consumption of spiders

In the 5 colonies studied here, out of the 16 prey species detected at least in 3% of the samples, 8 were orb-web building spiders of families Araneidae, Tetragnathidae and Uloboridae. Given how unusually other European bats prey on orb-weaving spiders (Whitaker 1988), such a consumption during the summer may alleviate the interspecific competition pressure. Bats can alter their foraging behaviour in the

presence of competing species to avoid competition (e.g. Andriollo et al. 2021; Novella-Fernandez et al. 2020; Salsamendi et al. 2012; reviewed in Salinas-Ramos et al. 2019). Spiders have been rarely regarded as bat prey until recently.

In Europe, several different bat species can consume spiders. *Myotis nattereri* sensu lato consumes them, as well as diurnal flies, during the autumn (Andreas et al. 2012a; Siemers and Swift 2006) and winter months (Hope et al. 2014). In the Iberian Peninsula, their sister species *M. crypticus* and *M. escalerai* also prey consistently on spiders (Novella-Fernandez et al. 2020). Spider remains have also been found in the faeces of *Myotis bechsteinii* and *Plecotus auritus*, for example (Andreas et al. 2012a). Additionally, with the use of multiple markers (Aldasoro et al. 2019), or generalist markers (Tournayre et al. 2020a) for DNA amplification, spiders are expected to appear as an occasional or regular prey item for other insectivorous bats as well. In fact, spiders have been recently reported to be an important food source for other bats of the genus *Myotis* in Europe and North America using molecular techniques for dietary studies, all of which forage in forests and catch their prey by gleaning (Hope et al. 2014; Maucieri and Barklay 2021; O'Rourke et al. 2021; Roswag et al. 2018).

In accordance with previous work on *M. emarginatus*' diet near the study area (Vallejo et al. 2019), we predicted that the consumption of spiders would be highest in May. However, the results presented in this paper show the opposite trend. Intrinsic differences between the colonies sampled



could explain the different responses detected. For example, one of the colonies from the previous study is in an area where cattle farming is the most abundant in the Basque Country. Therefore, alternate food sources like diurnal flies are likely to be much more abundant there than in the colonies from this study. On the other hand, for the present study, we significantly improved the sampling effort to focus on the seasonality of the diet, highlighting the importance of an adequate study design in the results, and their correct interpretation.

In the present study, *A. diadematus* was the most common prey species, and it was detected in 69% of the samples. It was mostly consumed in the late season, and in some weeks, it accounted for over half the wPOO of the total diet. Contrastingly, it was rarely consumed during May (wPOO %1.6). At this time, *Nuctenea umbratica* and *Metellina merianae*, other orb-web building spiders, were the most consumed prey species. Many orb-web building species show an increase in their abundance during late summer months or autumn, while others mature in the spring (Hsieh and Linsenmair 2012). *A. diadematus*, specifically, reaches maturity during late summer-early autumn in temperate zones, and at this time they would be most available to bats like *M. emarginatus* (Olive 1981). Given their similarity in morphology and lifestyle, it would be interesting to study whether all orb-web building spiders are functionally indistinguishable by *M. emarginatus* besides perhaps size (Arrizabalaga-Escudero et al. 2019; Divoll et al. 2022; Spitz et al. 2014). Therefore, we hypothesize that changes in diet variability and composition within this functional group should mirror the local availability of resources.

The wing morphology and echolocation of *M. emarginatus* suggest that orb-web-building spiders would be gleaned over orb webs or other surfaces. Dense habitats, mainly native broad-leaved and mixed forests, are the preferred foraging habitat of *M. emarginatus* in some colonies (Dekker et al. 2013; Zahn et al. 2010). However, it can also use conifer plantations and mixed forests in some areas of its distribution (Flaquer et al. 2008; Goiti et al. 2011). Additionally, the use of open grasslands, riparian forests and especially cattle sheds has also been reported in the northernmost part of the distribution area (Dietz et al. 2013; Zahn et al. 2010). Accordingly, flies are abundant in the *M. emarginatus* diet in these areas (e. g. Krull et al. 1991), which shows that the geographic differences reported in diet composition most likely correlate with differences in habitat use for foraging (Dietz and Pir 2021).

The affinity of *M. emarginatus* to dense foraging habitats in its meridional distribution area is usually linked to the predation on orb-web-building spiders (Goiti et al. 2011). Cluttered habitats, especially those with a high degree of vertical complexity, are essential for web-building spider communities to thrive, because they offer plenty of

web-building opportunities (Rubio and Moreno 2010; Ward and Lublin 1992). Additionally, diverse habitats also favour the diversity and abundance of spider prey (Dennis et al. 2015). Therefore, maintaining forests and other land patches with a complex vertical vegetation structure and a high degree of structural and vegetational heterogeneity is crucial to preserve attractive hunting grounds for colonies of *M. emarginatus*, and potentially other insectivorous bat species that, albeit to a lesser degree, also exploit this food resource.

### Consumption of flies

The second and third most consumed prey species were *Stomoxys calcitrans* and *Musca domestica* (Muscidae). Together, they made 20% wPOO between the end of May and June (Weeks 22–26). Their importance in the diet decreased as the season progressed, and the consumption of *A. diadematus* increased. Flies are the most important food source for *M. emarginatus* in Central Europe (Kervyn et al. 2012; Steck and Brinkmann 2006). In contrast, studies from Mediterranean areas report lower proportions of flies and instead describe a spider-based diet (Goiti et al. 2011; Vallejo et al. 2019).

Both *Stomoxys calcitrans* and *Musca domestica* are synanthropic species, associated with cattle farms. *Stomoxys calcitrans*, especially, is frequently found around bovine farms and cattle farms (González 2022). *Stomoxys calcitrans* is considered a significant pest for livestock and domestic animals. Both sexes are hematophagous, and their bite causes physical distress and injuries to animals, particularly when their abundance increases, and can act as a vector of severe diseases and pathogens (González 2022; González et al. 2022; Patra et al. 2018). Nowadays, no specific control measures are taken against *S. calcitrans* by farmers, as the basic knowledge about this species is still scarce (González et al. 2022).

Besides, both are diurnal flies and remain active only in the warmest hours of the day (González 2022), so *M. emarginatus* should glean them directly from surfaces. In Central Europe, radio tracking data has confirmed that *M. emarginatus* frequently visits cattle sheds at night to forage (Dekker et al. 2013; Krull et al. 1991; Zahn et al. 2010). These structures are key foraging habitats for *M. emarginatus*, as they provide a reliable food source, especially on colder nights (Dekker et al. 2013). Krull et al. (1991) did not report such a correlation between weather and the use of cattle sheds as foraging grounds, however. Besides *M. emarginatus*, other bat species that use forests as their foraging areas have occasionally been reported hunting flies inside cattle sheds (Ancilloto et al. 2021; Siemers et al. 2012). Additionally, the activity of open foragers increases around free-ranging cattle (Ancilloto et al. 2021).

DNA metabarcoding has allowed us to confirm that the consumption of *S. calcitrans* by *M. emarginatus* is frequent in the Basque Country, especially at the beginning of the summer. Therefore, it is very likely that it also forages in cattle farms in the Basque Country. Nonetheless, specific studies are needed to confirm this fact in the area. More research is needed to explore under what conditions bats exploit diurnal flies in cattle farms, especially in the southern area of the distribution of *M. emarginatus*, further away from the limit of their distribution, where more examples of a spider-dominated diet have been reported (Goiti et al. 2011; Vallejo et al. 2019). On the other hand, DNA Metabarcoding does not offer accurate quantitative data (Deagle et al. 2018), so estimating the potential of *M. emarginatus* colonies for biological control is challenging. Methods targeted to identify a single prey species (e.g. Baroja et al. 2021) are an interesting option to overcome some of the pitfalls of DNA metabarcoding in this regard.

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**Data availability** All data generated or analysed during this study are included in this published article in supplementary files 2 and 3. Additional raw data will be available from the corresponding author on reasonable request.

## Declarations

**Conflict of interest** The authors have no competing interests to declare that are relevant to the content of this article.

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