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Gleaning insectivorous bats eat seasonably in a West African rice farming landscape:

implications for ecosystem services

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Resumo

Os morcegos insetívoros desempenham um papel ecológico essencial ao atuarem como predadores naturais de artrópodes, contribuindo para a regulação das populações de invertebrados nos ecossistemas. A sua dieta generalista, aliada a estratégias alimentares diversificadas, permite uma rápida adaptação às flutuações nas presas. Esta plasticidade trófica é particularmente vantajosa em ambientes dinâmicos, como as paisagens agrícolas, onde a presença de pragas é diversificada e persistente ao longo do ano. Ao ajustarem a dieta às presas mais abundantes em cada estação, os morcegos mantêm uma pressão predatória contínua sobre diversos grupos de artrópodes, incluindo aqueles com impacto negativo nas culturas agrícolas.

Nos países em desenvolvimento como a Guiné-Bissau, onde a agricultura constitui uma das principais fontes de subsistência, segurança alimentar e rendimento económico, o impacto das pragas em culturas essenciais, como o arroz, pode ter consequências severas para as comunidades locais. Por isso, compreender de que forma predadores como os morcegos contribuem para mitigar esses impactos é essencial para a promoção de práticas agrícolas mais sustentáveis e para garantir a conservação dos serviços ecológicos prestados pela biodiversidade funcional.

Apesar do crescente reconhecimento do papel dos morcegos insetívoros como agentes de controlo biológico, ainda existe uma grande limitação no conhecimento dos hábitos alimentares de muitas espécies, sobretudo na África Ocidental. Neste contexto, o presente estudo surgiu com o propósito de caracterizar a dieta de duas espécies do género *Nycteris* (*Nycteris* cf. *hispida* e *Nycteris thebaica/gambiensis*) que ocorrem numa paisagem agrícola na Guiné-Bissau. Pretendeu-se compreender se a sua alimentação reflete padrões oportunistas e se é influenciada por variações sazonais na ocorrência das presas, incluindo as conhecidas por causar prejuízos agrícolas.

Deste modo, recorreu-se a técnicas de *DNA metabarcoding* e *next-generation sequencing* (NGS) para caracterizar a composição da dieta de *N.* cf. *hispida* e *N. thebaica/gambiensis*, tendo por base três objetivos: a) analisar se a composição da dieta de *N.* cf. *hispida* varia espacialmente (entre abrigos) e temporalmente; b) caracterizar a variação da dieta das duas espécies de *Nycteris* ao longo do período de amostragem e comparar essas variações; c) avaliar se ambas as espécies acompanham as flutuações sazonais de presas, incluindo as de potenciais pragas agrícolas.

Com base na literatura existente e no comportamento oportunista geralmente atribuído aos morcegos insetívoros, formulou-se a hipótese de que a composição da dieta variava tanto em função do tempo como do espaço. Antecipou-se que ambas as espécies apresentassem algum grau de diferenciação na dieta, com uma sobreposição parcial de presas, sugerindo mecanismos de partilha de recursos que possibilitassem a sua coexistência. Por fim, pressupôs-se que os morcegos ajustariam a sua dieta consoante as mudanças sazonais na ocorrência das presas, aumentando o consumo das espécies mais frequentes, incluindo potenciais pestes.

O trabalho de campo decorreu entre junho de 2022 e dezembro de 2023 na aldeia de Djalicunda, na região de Oio, no norte da Guiné-Bissau, onde foram recolhidas amostras fecais de dois abrigos previamente identificados como locais de refúgio de colónias de *Nycteris*. A identificação das presas consumidas foi realizada através da técnica de *metabarcoding*, com amplificação do gene mitocondrial COI e posterior sequenciação por NGS. As sequências obtidas foram processadas e agrupadas em unidades taxonómicas operacionais (OTUs) e depois comparadas com bases de dados de referência,

como BOLD e NCBI, para a atribuição do menor nível taxonómico possível. As OTUs presentes numa única amostra (*singletons*) foram excluídas. Para cada amostra, a proporção de cada táxon foi ponderada pelo número total de taxa presentes, o que resultou na proporção ponderada de ocorrência (wPOO).

As análises estatísticas foram realizadas com recurso ao R (versão 4.4.3). Recorreu-se a técnicas multivariadas, incluindo *non-metric multidimensional scaling* (NMDS) para avaliar a composição da dieta ao longo do tempo e entre abrigos, *analysis of similarities* (ANOSIM), e *permutational multivariate analysis of variance* (PERMANOVA) para analisar os efeitos espaciais e temporais na dieta. Foi também realizada uma *indicator species analysis* para identificar associações significativas entre grupos de presas e combinações específicas de abrigo e mês. Para analisar as tendências de consumo de presas por grupo taxonómico e por OTUs, ajustaram-se *generalize additive models* (GAM) para cada predador, permitindo modelar padrões não-lineares de variação ao longo do tempo. Além disso, para explorar se estes morcegos acompanham a variação sazonal das presas mais frequentes, analisou-se o consumo das 10 OTUs com maior ocorrência, juntamente com OTUs identificadas como pragas agrícolas, visualizando as tendências da variação do wPOO ao longo dos meses através de curvas de suavização LOESS (*locally estimated scatterplot smoothing*).

No total, foram analisadas 166 amostras fecais, tendo sido identificadas 2233 ocorrências de artrópodes, correspondendo a 488 OTUs, distribuídas por 100 famílias e 15 ordens taxonómicas. Os resultados mostraram que a dieta de *N.* cf. *hispida* foi mais influenciada pela variação temporal, do que pela localização do abrigo, indicando uma forte resposta sazonal na ocorrência de presas. Ainda assim, foram identificados conjuntos específicos de presas associados a combinações de abrigo e mês, refletindo um efeito conjunto do espaço e do tempo na composição da dieta.

A análise com modelos GAM revelou padrões distintos entre os dois morcegos. Em média, *N.* cf. *hispida* consumiu Coleoptera mais frequentemente, enquanto *N. thebaica/gambiensis* consumiu mais Lepidoptera. Para além disso, *N.* cf. *hispida* exibiu variações temporais significativas no consumo de várias ordens (incluindo Coleoptera, Orthoptera e Lepidoptera), enquanto *N. thebaica/gambiensis* apenas variou significativamente no consumo de Coleoptera. As curvas LOESS mostraram flutuações marcadas das OTUs com picos coincidentes com a estação das chuvas e períodos de crescimento agrícola, nomeadamente para presas como *Spodoptera littoralis*, *Achaea ezea* e *Trinervitermes togoensis*, todas reconhecidas como pragas agrícolas. Algumas pragas, como *Macrotermes bellicosus*, *Orseolia oryzae* e *Anarsia* sp., foram consumidas de forma consistente ao longo dos meses, o que pode indicar um possível serviço contínuo de controlo biológico.

Estes resultados reforçam a ideia de que *N.* cf. *hispida* tem uma dieta flexível, ajustando-se às alterações temporais dos recursos. O menor efeito da localização dos abrigos na composição dieta poderá estar associado à similaridade da paisagem agrícola em redor dos locais de amostragem e à sobreposição dos habitats de forrageamento utilizados pelas colónias. A diferenciação taxonómica de presas entre os dois predadores poderá refletir uma repartição de recursos, provavelmente relacionada com tipos de forrageamento distintos: *N.* cf. *hispida*, poderá recorrer preferencialmente a estratégias de *gleaning*, capturando presas presentes em superfícies, enquanto *N. thebaica/gambiensis* poderá adotar estratégias mais relacionadas com *fly-catching* optando por presas mais aéreas. Para além disso, diferenças morfológicas associadas à forma e funcionamento das asas, como o *aspect ratio* e a carga alar, também poderão influenciar esta repartição, uma vez que influenciam a manobrabilidade e a seleção de habitat.

A plasticidade alimentar observada em ambos os morcegos evidencia a sua capacidade de adaptação a uma paisagem agrícola dinâmica. O consumo frequente de presas consideradas prejudiciais para as culturas sugere que estes morcegos poderão desempenhar um serviço ecológico relevante no consumo de pragas, com benefícios diretos para os sistemas agrícolas locais.

Em suma, este estudo demonstra que *Nycteris* cf. *hispida* e *N. thebaica/gambiensis* são predadores generalistas com estratégias alimentares distintas, capazes de ajustar a sua dieta em resposta à variação espacial e temporal de recursos. A sua relevância enquanto agentes naturais de controlo biológico em paisagens agrícolas africanas deve ser reconhecida e integrada nas políticas de conservação da biodiversidade e nas práticas de gestão agrícola. A proteção destas populações de morcegos, bem como a manutenção dos seus habitats são cruciais para a promoção de serviços importantes para o ecossistema e para o desenvolvimento de uma agricultura mais resiliente e sustentável.

Palavras-chave: *Nycteris*, Guiné-Bissau, controlo biológico, *metabarcoding*, dieta

Abstract

Insectivorous bats are key predators of arthropods, yet their ecological roles in African agroecosystems remain underexplored. This study investigates the dietary variation of two slit-faced bats (*Nycteris* cf. *hispida* and *Nycteris thebaica/gambiensis*) inhabiting a rural landscape in Guinea-Bissau, West Africa. Understanding the feeding patterns of these bats is essential for assessing their contribution to natural pest control and biodiversity conservation.

Using DNA metabarcoding and next-generation sequencing, we examined spatial and temporal variations in prey consumption to assess dietary overlap, flexibility and potential pest suppression. Specifically, we focused on the following objectives: a) to assess whether the diet composition of *N.* cf. *hispida* varies spatially (between roosts) and temporally; b) to characterise how the diet of the two *Nycteris* species varies throughout the sampling period and to compare these patterns; c) to evaluate whether both predators track seasonal changes in prey, particularly potential agricultural pests.

We analysed 166 faecal samples that revealed 488 distinct OTUs. *Nycteris* cf. *hispida* exhibited pronounced temporal variation in its diet, likely reflecting changes in arthropod occurrence, while *N. thebaica/gambiensis* showed less pronounced seasonal shifts. Despite shared roosts, differences in prey composition and foraging strategies suggest partial niche differentiation, with *N.* cf. *hispida* favouring ground-dwelling invertebrates and *N. thebaica/gambiensis* exploiting more aerial arthropods. Both bats consumed potential agricultural pests, including *Spodoptera littoralis*, *Macrotermes bellicosus* and *Orseolia oryzae*.

These results highlight the dynamic foraging response of *Nycteris* bats to changes in prey availability and their potential role in controlling insect pests in West African rice systems. This study contributes to a growing body of evidence supporting insectivorous bats' ecological and economic value and underscores the importance of conserving bat populations in rural landscapes.

Keywords: *Nycteris*, Guinea-Bissau, biological control, metabarcoding, diet

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List of Abbreviations

ANOSIM – Analyses of similarity

BOLD – Barcode of Life Data System

COI – Mitochondrial cytochrome oxidase I

DNA – Deoxyribonucleic acid

FAO – Food and Agriculture Organization of the United Nation

GAM – Generalised additive models

GAMM – Generalised Additive Mixed Models

IUCN – International Union for Conservation of Nature

LOESS – Locally estimated scatterplot smoothing

NCBI – National Center for Biotechnology Information

NGS – Next generation sequencing

NMDS – Non-metric multidimensional scaling

OTU – Operational taxonomic unit

PCR – Polymerase chain reaction

PERMANOVA – Permutational multivariate analysis of variance

wPOO – Weighted proportion of occurrence

1. Introduction

Insectivorous bats consume a wide variety of arthropods, eating up to 70–100% of their body weight nightly (Kunz et al., 2011), making them crucial top-down regulators of arthropod populations (Kalka et al., 2008; Maas et al., 2016). Their generalist diets and diverse foraging strategies allow a quick response to changes in prey availability, promoting food web and ecosystem stability (Maine & Boyles, 2015b; McCracken et al., 2012). Many species are also opportunists, adjusting their diet to local and seasonal shifts in arthropod communities (McCracken et al., 2012).

Slit-faced bats (family Nycteridae) are distinguished by a longitudinal slit between the nasal leaves, bordered by fleshy protuberances that likely assist in echolocation (Kingdon, 2013; Monadjem et al., 2010). This family comprises a single genus, *Nycteris*, with 14 out of 16 recognised species distributed in continental Africa and the Arabian Peninsula and two in southern Asia (Demos et al., 2019). In Guinea-Bissau, five species are known to occur: *N. hispida*, *N. thebaica*, *N. macrotis*, *N. gambiensis* and *N. grandis* (IUCN, 2025).

Nycteris are small to medium-sized bats (32–65 mm). They typically roost alone, in pairs, or in small family groups, in semi-dark shelters, including caves, mines, road culverts, hollow logs, tree branches and even human dwellings (Kingdon, 2013; Monadjem et al., 2010). They may alternate between day and night roosts for feeding and/or resting (Fenton et al., 1990; Monadjem, 2005), with reported commuting distances ranging up to 2.2 km in *N. grandis* (Fenton et al., 1990), the genus's largest species, and 1.1 km in *N. thebaica* (Monadjem et al., 2009). Their broad, rounded wings and low wing loading support slow, highly manoeuvrable flight, enabling them to hover, turn tight spaces and forage near the ground or within dense vegetation (Kingdon, 2013; Norberg & Rayner, 1987).

Nycteris are clutter foragers and gleaners, capturing prey from surfaces using low-intensity echolocation calls (Kingdon, 2013; Monadjem et al., 2010; Schnitzler & Kalko, 2001). In these environments, prey echoes are masked by background clutter, so they rely on prey-generated sounds, assisted by their large ears and acute hearing (Bayefsky-Anand, 2005; Bowie et al., 1999; Fenton et al., 1983). Some species also alternate between continuous and perch flight strategies to enhance prey detection (Aldridge et al., 1990; Fenton et al., 1990). Their diet is remarkably diverse, primarily consisting of insects and arachnids (Bowie et al., 1999; LaVal & LaVal, 1980; Monadjem et al., 2010; Taylor et al., 2011). Certain species are reported to prefer large, abundant and often non-volant or stationary prey that are easy to detect and capture (LaVal & LaVal, 1980). Given their foraging ecology and diverse diets, *Nycteris* species may provide important ecosystem services in human-modified landscapes, including agricultural areas.

Agriculture remains vital for food security and rural livelihoods, especially in developing regions (Pawlak & Kołodziejczak, 2020). Rice (*Oryza* spp.), a staple for over half the world's population (Muthayya et al., 2014), is highly vulnerable to crop pests (Oerke, 2006). In West Africa, pest outbreaks pose a significant threat to rice yields and food security (Adjah et al., 2022; Nwilene et al., 2013). Reducing pest impacts is therefore crucial to improving production and supporting smallholder farmers (Ratto et al., 2022). Studying the diets of insectivorous bats can help assess their role in natural pest control and guide sustainable management strategies.

In this study, we used DNA metabarcoding and next-generation sequencing (NGS) to characterise the diet of two *Nycteris* species (*Nycteris* cf. *hispida* and *Nycteris thebaica/gambiensis*) in a rural landscape in Guinea-Bissau. By analysing faecal samples across different months, we aim to characterise prey composition and assess whether their dietary patterns reflect opportunism or seasonal foraging.

Specifically, we addressed the following questions:

a) Does the diet composition of *N.* cf. *hispida* vary spatially (between roosts) and temporally?
b) How does the diet of the two *Nycteris* species vary throughout the sampling period and how do these variations compare?
c) Do both predators track seasonal prey, particularly potential agricultural pests?

We hypothesise that diet composition will vary both spatially and temporally, reflecting local prey occurrence and seasonal shifts in arthropod communities (Clare et al., 2011; Tiede et al., 2020). Despite sharing a roost and potentially overlapping foraging ranges, we expect partial dietary differentiation between species (Arrizabalaga-Escudero et al., 2018; Blanch et al., 2023; Zhu et al., 2024). We also predict that both bats will track seasonal prey fluctuations, leading to increased consumption of frequently occurring prey, including agricultural pests (Kunz et al., 2011).

2. Methods

2.1. Study area

This study was conducted in the village of Djalicunda in the Mansabá sector, located in the Oio region in the north of Guinea-Bissau (Figure 2.1). The climate of this region is defined by two distinct seasons: a rainy season, which extends from June to November, and a dry season from December to May. The mean annual temperature ranges from 25.9 to 27.1°C, while the annual rainfall varies from 1200 to 1400 millimetres (Catarino et al., 2008; Mendes et al., 2025).

We studied two species of slit-faced bats, *Nycteris* cf. *hispida* and *Nycteris thebaica/gambiensis*, in colonies roosting in two abandoned houses: roost A and roost B (see Figure 2.1), located approximately 660 metres apart. The surrounding landscape, within a radius of 1.1 km (equivalent to the foraging range of *N. thebaica* (Monadjem et al., 2009)), was composed of a mosaic of agricultural crops, including rice paddies and cashew orchards, wooded and open areas, and some humanised areas. The landscape was mapped and classified using QGIS v3.28 (QGIS Development Team, 2024). Both roosts initially harboured several dozen individuals. Roost A was occupied year-round by a multispecies assemblage, including *N.* cf. *hispida*, *N. thebaica/gambiensis*, and occasionally *Hipposideros ruber*; it was also confirmed as a maternity site for *N.* cf. *hispida*. Roost B was seasonally occupied, typically by a monospecific colony of *Nycteris* cf. *hispida.*

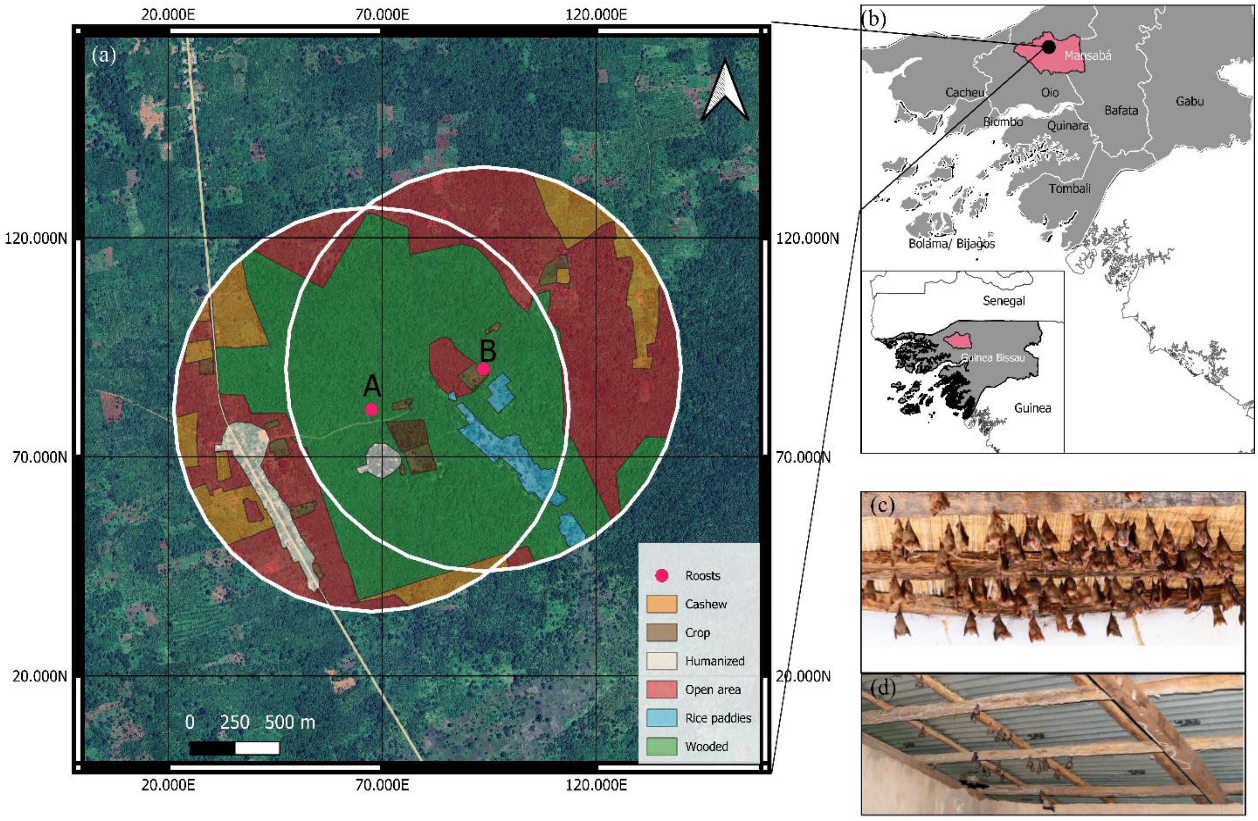

Figure 2.1. Study area showing the two bat roosts and surrounding landscape within a radius of 1.1 km (a). Insets show the location of Guinea-Bissau and the Mansabá sector (b), and photographs of roost A (c) and roost B (d).

2.2. Sample collection

Between early June 2022 and early December 2023, faecal samples were collected from both roosts to identify the consumed prey. Sampling took place in June, September, October, November and December of 2022, and in March, May, June, July, September, October, November and December of 2023. At each roost, a 1m x 1m plastic sheet was placed beneath the colony for three consecutive days. 15 samples were collected from the plastic and immediately preserved in 2 ml tubes filled with 96% ethanol. This procedure was repeated every fortnight.

The selection of sampling months and the fortnightly frequency were designed to capture potential dietary variation throughout the year and reflect key phases of the local rice cultivation cycle. In the study region, rice is typically sown in June, followed by rapid vegetative growth through July to September, maturation from October to November and harvest sometimes extending into January. By spreading sample collections across the transition from dry to wet season (May to June), vegetative and reproductive growth (July to mid-November) and harvest periods (December), the protocol enabled the detection of seasonal shifts in arthropod occurrence potentially linked to agricultural activities and rice phenology.

2.3. DNA Extraction, Amplification and Sequencing

DNA extraction and subsequent processing were conducted manually on a total of 166 faecal samples. The number of individuals and pellets analysed was determined by balancing the need to maximise the accuracy of diet estimates with the associated costs (Mata et al., 2019). The protocol for DNA extraction followed the method described by Gonçalves et al. (2024). Initially, 650 µL of lysis buffer (0.1 M Tris-HCl, 0.1 M EDTA, 0.01 M NaCl, 1% N-lauroylsarcosine, pH 7.5-8; (Maudetr et al., 2002) was added to each sample. This was followed by homogenisation using a sterile spatula, vortexing and incubation in a dry bath at 56°C for 30 minutes. The samples were vortexed again for one minute and then centrifuged at 12,000 × g for 30 seconds. After centrifugation, up to 500 µL of the resulting interphase was transferred to a new tube and 25 µL of OB Protease was added. The subsequent steps adhered to the protocol of the E.Z.N.A. Tissue Kit (Omega Bio-Tek, Georgia, USA), with the exception that DNA was eluted twice in 50 µL, resulting in two separate extracts.

The DNA of the preyed-upon arthropods was amplified using the FwhF2-R2n COI primers (Vamos et al., 2017), which were modified to include Illumina adapters. The effectiveness of these primers has been demonstrated in amplifying a diverse range of arthropods, including those with degraded DNA (da Silva et al., 2024; Elbrecht et al., 2019; Mata et al., 2021). The PCR reaction mixture consisted of 5 µL of Qiagen Multiplex Master Mix, 0.3 µL of each primer at a concentration of 10 nmol/L, 3.4 µL of water, and 1 µL of extracted DNA. The cycling conditions included an initial denaturation step of 15 minutes at 95°C, followed by 40 cycles of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 50°C, and elongation for 30 seconds at 72°C, concluding with a final elongation step of 10 minutes at 72°C.

The identifications in the field were confirmed by amplifying a small fragment of the COI gene using FwhF1-R1 primers (Vamos et al., 2017). This step was crucial in validating the presence of cryptic species, as the Fwh2 primers often fail to detect vertebrate taxa. The PCR conditions for this amplification mirrored those used for prey DNA. Following amplification, the PCR products were diluted at a ratio of 1:4. A second PCR was conducted to introduce 7 bp indices along with Illumina P5 and P7 sequencing adapters. The PCR mixture and cycling parameters closely resembled those of the initial PCR, aside from utilizing the KAPA HiFi HotStart ReadyMix (Roche, Basel, Switzerland) and performing eight cycles with an annealing temperature set to 55°C. The indexed PCR products were purified with Agencourt AMpure XP beads (Beckman Coulter, Brea, California, USA) at a ratio of 1:0.8. Subsequently, the purified products were quantified using an Epoch microplate spectrophotometer (Agilent, California, USA), diluted to a concentration of 15nM and organized by manufacturer. Each library was quantified individually using qPCR (KAPA Library Quant Kit qPCR Mix; Roche), re-diluted to 4nM and sequenced on a MiSeq with a target depth of 30k reads for Fwh2 and 4k reads for Fwh1.

2.4. Bioinformatic analysis

The software Flash was utilized to merge paired reads (Magoč & Salzberg, 2011). Subsequently, Obitools commands—including ‘ngsfilter,’ ‘obiuniq,’ and ‘obigrep’ (Boyer et al., 2016) —were employed to eliminate primer sequences, dereplicate reads within each sample and discard singletons.

VSEARCH was then applied, utilizing the ‘cluster_unoise’ command to denoise reads, the ‘—uchime3_denovo’ command to remove chimeric sequences, the ‘—cluster_size’ command for clustering at 99% similarity and the ‘—usearch_global’ option to map reads back to the retained operational taxonomic units (OTUs) (Rognes et al., 2016). Following this, LULU was employed to merge similar OTUs (>84% similarity) that exhibited high levels of co-occurrence (>95%; Frøslev et al., 2017). OTUs not conforming to the expected length (within 205 ± 3 bp; Vamos et al., 2017) were excluded from further analysis. To account for potential contamination, the number of reads from extraction and PCR negative controls was subtracted from the associated samples. The resulting OTUs were then compared against the BOLD and NCBI databases to achieve identification at the most refined taxonomic level possible. In cases where OTUs exhibited similar identities across multiple species, genera, or other taxonomic ranks, the broadest level of classification was chosen. Finally, OTUs were classified as dietary (e.g., most arthropods) or non-dietary (e.g., predators, fungi, internal and external parasites).

DNA metabarcoding has some limitations, particularly the risk of detecting secondary ingestion. This phenomenon can occur when arthropod predators consume prey species, which are subsequently ingested by bats, leading to the detection of the prey DNA (da Silva et al., 2019; Deagle et al., 2019). However, since the DNA from the gut contents of the prey is typically present in smaller quantities and is more susceptible to degradation, the likelihood of successful amplification during PCR is reduced (Mata et al., 2021). To mitigate the impacts of secondary ingestion, additional filtering was employed, including the exclusion of samples with fewer than 100 dietary reads and the removal of dietary operational taxonomic units (OTUs) that constituted less than 1% of the total reads per sample (Deagle et al., 2019).

2.5. Statistical Analysis

All statistical analyses were conducted using R statistical software version 4.4.3 (R Core Team, 2025), with visualisations produced using the *ggplot2* package (Wickham, 2016). Additional packages are cited where relevant. Diet singleton OTUs—prey taxa found in only one sample—were excluded from the analysis. For each sample, the proportion of each prey taxon was weighted by the total number of taxa, resulting in weighted proportion of occurrence (wPOO). While the wPOO metric accounts for relative prey frequencies within samples, it does not correct for potential biases introduced by differential prey DNA detectability, digestion rates, or amplification efficiency (Deagle et al., 2019). Therefore, results should be interpreted as reflecting relative detection frequencies rather than absolute prey biomass or abundance.

To examine spatial and temporal patterns in the diet of *N.* cf. *hispida* across the two roosts, we used Non-Metric Multidimensional Scaling (NMDS) based on the Jaccard dissimilarity. The dataset was analysed in two dimensions (k = 2) and included samples from December 2022 and May 2023, when both roosts were occupied by *N.* cf. *hispida*. Samples were grouped by roost and month (“A-May”, “A-December”, “B-May”, “B-December”). NMDS was performed using the *metaMDS* function from the *vegan* package (Oksanen et al., 2022). Dietary differences between groups were tested with an analysis of similarities (ANOSIM), based on the Jaccard dissimilarity with 999 permutations. To assess the influence of roost location, month and their interaction, we applied PERMANOVA (*adonis2*, *vegan*), also using Jaccard dissimilarity with 999 permutations. Indicator species analysis was used to detect prey OTUs associated with specific groups, using the *multipatt* function from the *indicspecies* package (De Cáceres & Legendre, 2009).

To analyse the annual variation in the diet of the two *Nycteris* species, we modelled wPOO values for arthropod orders with >30 detections. Values were log-transformed and analysed using Generalised Additive Models (GAMs) with a quasibinomial distribution. Initially, Generalised Additive Mixed Models (GAMMs) were fitted including year as a random effect; however, as the estimated variance component for year approached zero, indicating a negligible effect, we proceeded with GAMs without random effects. These models were implemented using the *gam* function from the *mgcv* package (Wood, 2025). The analysis was repeated at the prey OTU level to further explore seasonal and prey species-specific dietary trends, focusing on the 10 most frequent prey OTUs. For all models, the goodness-of-fit was assessed using the coefficient of determination (R-sq adj.), indicating the proportion of variation in the response variable explained by the predictors.

In both cases, wPOO was modelled as a smooth function of the sampling month. This approach allowed for the detection of non-linear trends in dietary variation and was performed separately for each *Nycteris* species to identify distinct seasonal patterns in prey consumption. Additionally, we included each *Nycteris* species as a fixed factor to account for predator species-level differences in diet.

To investigate the seasonal dynamics in prey consumption, we analysed wPOO for the 10 most frequently consumed OTUs overall, along with key pest taxa known from the literature and confirmed to occur frequently in the diet with >10 detections (a detailed description of these pests is available in Table S2), to track their prey consumption. This selection resulted in a total of 17 OTUs. This selection was designed to focus on ecologically meaningful and frequently detected prey items, facilitating robust temporal trend analysis. We then examined the variation in the wPOO of each prey item across sampling months. The *ggplot2* package was employed to visualise these trends, with a locally estimated scatterplot smoothing (LOESS) curve with a span of 0.6 to depict detailed fluctuations in prey composition over time.

3. Results

We detected two *Nycteris* OTUs in our faecal samples: one matched consistently *N.* cf. *hispida* (~99% similarity) and is referred to as such; the other matched *N. thebaica* (97.5%), below our species-level threshold, with no sequences for *N. gambiensis* available in public databases. Although the few captured individuals matched *N. gambiensis* morphologically (bifid upper incisors, forearm 39.7 mm, tail 48 mm, ear 25 mm), the two species are difficult to distinguish in West Africa, where they are sympatric and share similar external and cranial characteristics (Kingdon, 2013). We therefore conservatively refer to this OTU as *N. thebaica/gambiensis*. FASTA sequences are available in the Supplementary Materials (Table S1).

A total of 166 faecal samples were analysed, with 107 from *Nycteris* cf. *hispida* and 59 from *Nycteris thebaica/gambiensis*. The samples from *N.* cf. *hispida* correspond to the entire sampling period, while the samples from *N. thebaica/gambiensis* date from September to December. Of the 124 samples collected from roost A, 65 were from *N.* cf. *hispida* and 59 from *N. thebaica/gambiensis*. The remaining 42 samples, all from *N.* cf. *hispida*, were collected from roost B.

The DNA metabarcoding analysis revealed 2233 occurrences of arthropod prey across three different classes: Arachnida (192), Chilopoda (8) and Insecta (2033). These belonged to 488 distinct OTUs, comprising 100 families and 15 orders. In total, 374 OTUs were detected in *N.* cf. *hispida* samples and 231 in *N. thebaica/gambiensis* samples, with 117 OTUs shared between both species. Only 15.6% of the prey OTUs were identified at the species level.

3.1. Spatial and temporal overlap in the diet of *N.* cf. *hispida*

The NMDS analysis (stress value = 0.144) showed partial overlap in the diet composition of *Nycteris* cf. *hispida*, with the colonies in both roosts consuming similar prey, particularly in December (Figure 3.1). However, this spatial overlap did not extend temporally, as the diet similarity was markedly lower between May and December. The ANOSIM results support this finding, showing a significant moderate-to-strong separation in the diet composition of the four groups, with an R-value of 0.621 (p = 0.001). The PERMANOVA results showed that both month (R^2 = 20.5%, p = 0.001) and roost location (R^2 = 4.1%, p = 0.002) had a significant effect on prey composition, with month exhibiting the strongest influence. The significant interaction between these two variables (R^2 = 4.2%, p = 0.003) indicates that the effect of month on diet composition differed between roosts (Table S3).

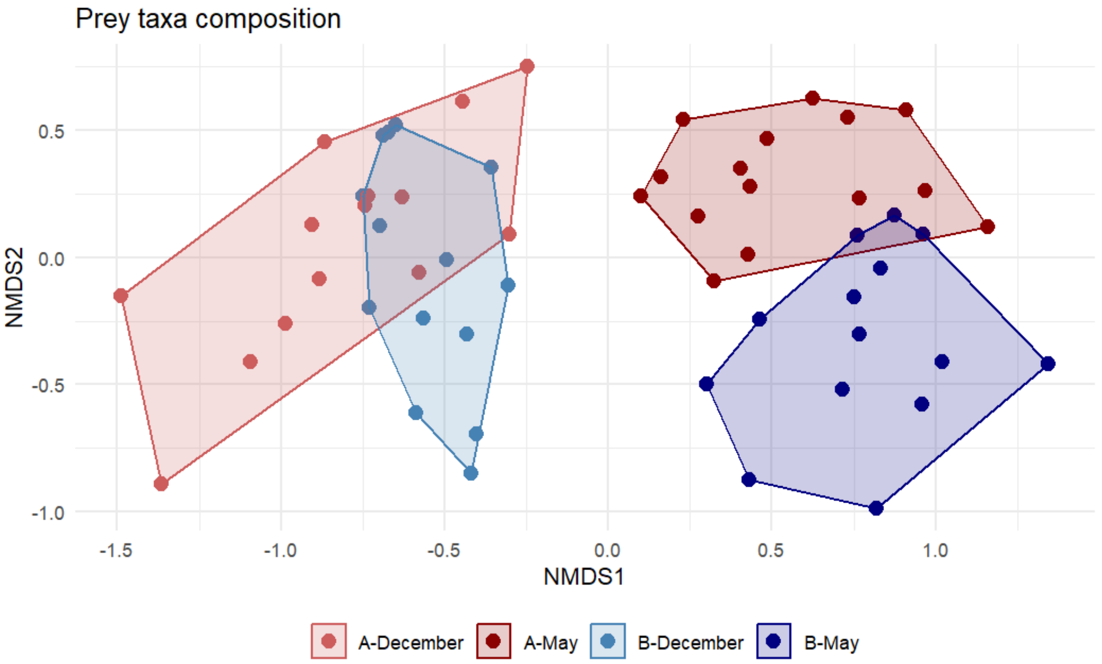

Figure 3.1 Non-metric dimensional scaling (NMDS) representing the composition of prey taxa (considering prey taxa occurrence) in the two roosts of *N.* cf. *hispida* (A and B) for May and December.

The temporal and spatial variation of the diet of *N.* cf. *hispida* was driven by prey taxa significantly associated with specific roost-month groups (Table S4). Multilevel pattern analysis revealed that in December, *Trichonephila fenestrata,* Scarabaeidae otu33, Araneae otu61 and Tettigoniidae otu97 were strongly linked to both roosts, indicating common prey or overlapping foraging areas during this period. In contrast, Ectobiidae otu4, Gryllidae otu62, *Elaeidobius pilimargo*, *Spodoptera littoralis,* were significantly associated with May. A subset of taxa (15.3%), including *Episparis* otu1123, *Elaeidobius* *kamerunicus*, *Autocharis fessalis* and *Neoscona penicillipes*, were exclusive to single roost-month groups. Nevertheless, most prey taxa (75.8%) were shared across months or roosts.

3.2. Annual variation in prey consumption

The GAM results revealed shifts in consumption of the nine most consumed arthropod orders between *N.* cf. *hispida* and *N. thebaica/gambiensis* throughout the sampling periods (Figure 3.2, Table 3.1). GAMs showed that the two predators differed significantly in the average consumption frequency of Coleoptera and Lepidoptera (Table S5). *N.* cf. *hispida* significantly consumed more Coleoptera, while *N. thebaica/gambiensis* consumed Lepidoptera at a significantly higher level.

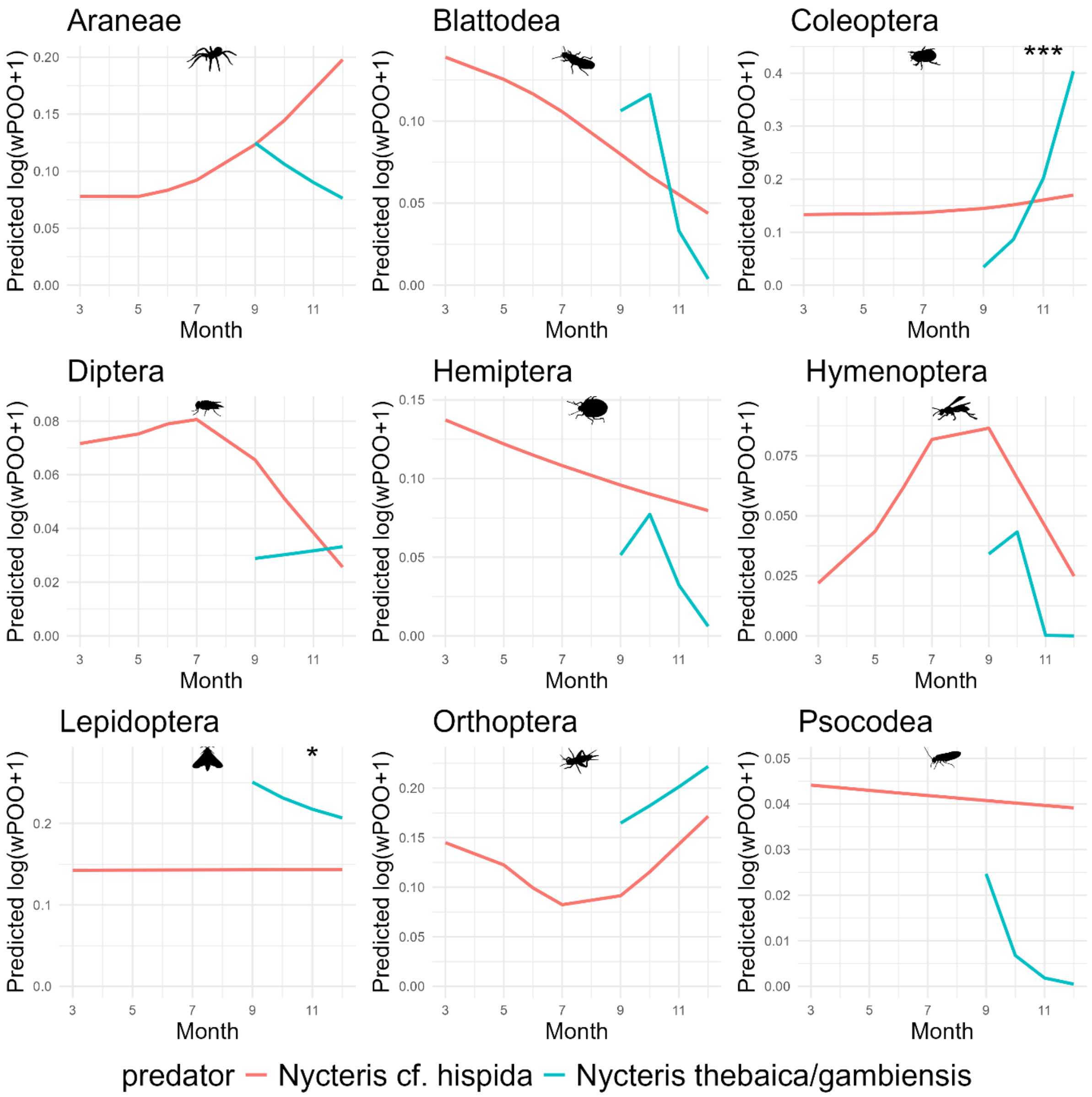

Figure 3.2. Predicted proportional occurrence (log-transformed wPOO+1) of arthropod orders in the diet of *Nycteris* cf. *hispida* (red) and *Nycteris thebaica/gambiensis* (blue) across sampled months using GAM modelling. Significant differences in the consumption of arthropod orders between bat species are indicated by *** ($p < 0.001$) and * ($p < 0.05$). Note that the scale of the Y axis differs between the graphs. Please refer to Table 3.1 and Table S5 for full model statistics.

Temporal variation has a significant effect on the diet composition of both bat species (Table 3.1). *N.* cf. *hispida* showed significant temporal variations in the consumption of Araneae, Blattodea, Hemiptera, Hymenoptera and Orthoptera. In contrast, *N. thebaica/gambiensis* exhibited a significant temporal variation only in the consumption of Coleoptera. Diptera, Lepidoptera and Psocodea consumption did not vary significantly over time in either bat species.

The R-sq adj. values varied among arthropod groups (Table 3.1), typically indicating modest explanatory power. For example, the GAM for Coleoptera consumption by *N. cf. hispida* achieved an R-sq adj. of 0.261, indicating that about 26% of the variation in Coleoptera consumption was explained by the model predictors. Other models for orders such as Blattodea and Hymenoptera had R-sq adj. values around 0.11–0.12, while some groups exhibited even lower values. These values demonstrate that, although considerable residual variation, a relevant fraction is captured by the predictors considered.

Table 3.1. Approximate significance of the smooth terms from the GAM model assessing predation by both *Nycteris* species on different arthropod orders across the sampling period. Differences in prey consumption over time are indicated by significance levels: *** < 0.001; **< 0.01; * < 0.05.

		Edf	Ref.df	F	p-value	Signif.	R-sq.(adj)
Araneae	*N.* cf. *hispida*	1.762	2.152	7.605	0.000499	***	0.0923
	N. thebaica/gambiensis	1.000	1.000	0.660	0.417739		
Blattodea	*N.* cf. *hispida*	1.601	1.921	6.685	0.00442	**	0.112
	N. thebaica/gambiensis	1.843	1.976	1.373	0.22154		
Coleoptera	*N.* cf. *hispida*	1.442	1.738	1.38	0.167		0.261
	N. thebaica/gambiensis	1.000	1.000	43.55	<2e-16	***	
Diptera	*N.* cf. *hispida*	2.203	2.672	3.094	0.0638		0.0894
	N. thebaica/gambiensis	1.000	1.000	0.019	0.8904		
Hemiptera	*N.* cf. *hispida*	1.000	1.000	4.297	0.0398	*	0.113
	N. thebaica/gambiensis	1.815	1.966	0.732	0.4424		
Hymenoptera	*N.* cf. *hispida*	2.515	3.041	4.060	0.00806	**	0.119
	N. thebaica/gambiensis	1.852	1.978	0.099	0.91235		
Lepidoptera	*N.* cf. *hispida*	1.000	1.000	0.001	0.981		0.114
	N. thebaica/gambiensis	1.075	1.145	0.499	0.591		
Orthoptera	*N.* cf. *hispida*	2.901	3.347	3.972	0.0148	*	0.106
	N. thebaica/gambiensis	1.000	1.000	1.001	0.3184		
Psocodea	*N.* cf. *hispida*	1	1	0.074	0.7857		0.0537
	N. thebaica/gambiensis	1	1	2.995	0.0854		

The analysis of the most prevalent prey at the OTU level also yielded low adjusted R^2 values, with only three OTUs explaining around or more than 20% of the variance (Table 3.2). Among shared prey, only the average consumption of Scarabaeidae otu11 differed between species, with *N.* cf. *hispida* consuming it more (Table S6). Temporal variation in prey intake was also evident at this level (Figure 3.3, Table 3.2), with both predators showing significant temporal patterns in the consumption of Scarabaeidae otu11 and otu33, both showing a pattern of increased occurrence toward the end of the year. In addition, *N.* cf. *hispida* showed significant temporal variation in the consumption of *Calycopis* sp. otu13, which peaked at the onset of the rains and Araneae otu61, which peaked toward the end of the year.

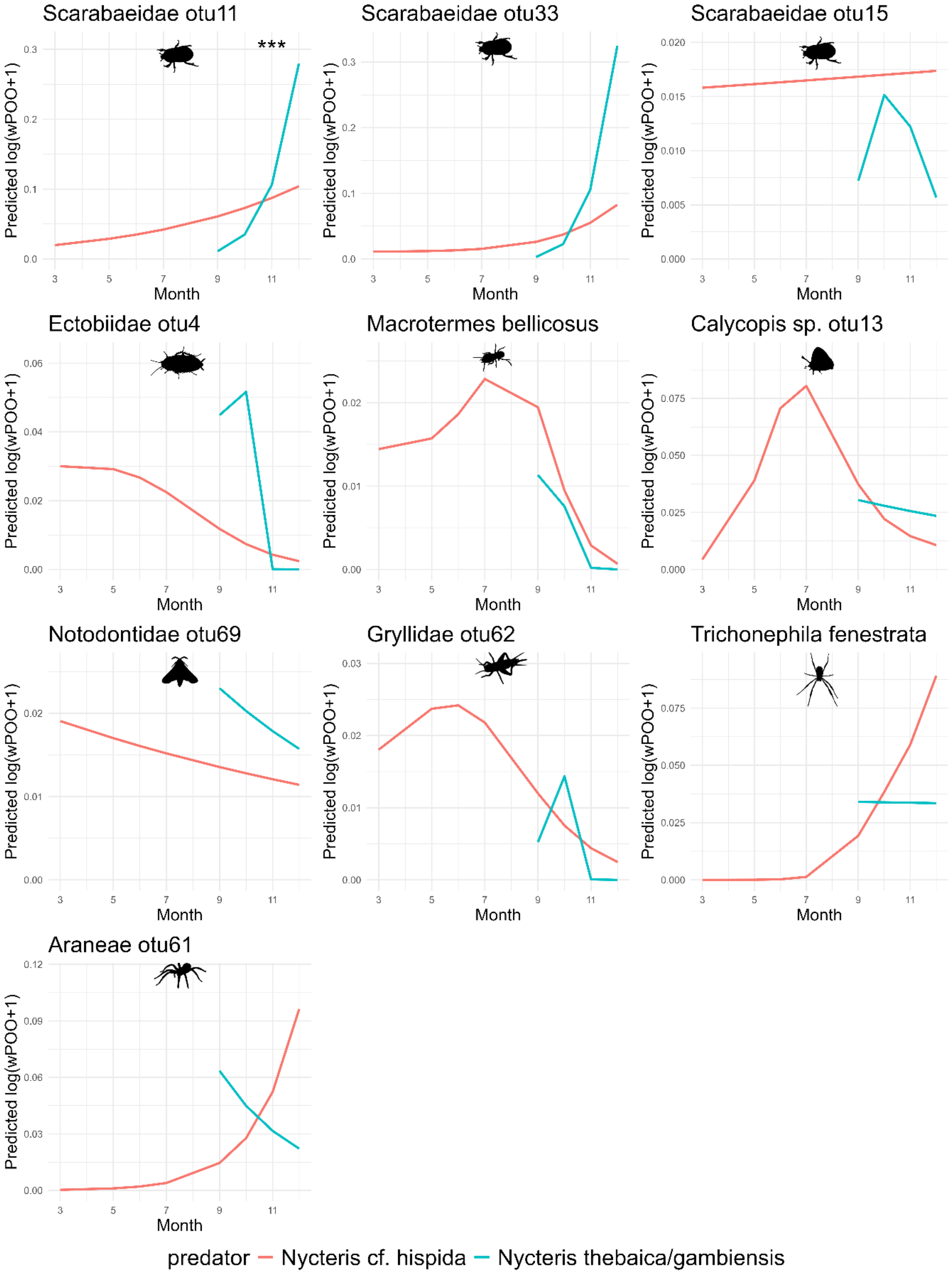

Figure 3.3. Predicted proportional occurrence (log-transformed wPOO+1) of the 10 most frequent arthropod OTUs in the diet of *Nycteris* cf. *hispida* (red) and *Nycteris thebaica/gambiensis* (blue) across sampled months modelled using GAMs. Scarabaeidae otu11 was the only prey taxon with significant difference in consumption between bat species, as indicated by * ($p < 0.05$) and *** ($p < 0.001$). Note that the scale of the Y axis differs between the graphs. Please refer to Table 3.2 and Table S6 for full model statistics.

Table 3.2. Approximate significance of the smooth terms from the GAM model assessing predation by both *Nycteris* species on different arthropod OTUs across the sampling period. Differences in prey consumption over time are indicated by significance levels: *** < 0.001; ** < 0.01; * < 0.05.

		Edf	Ref.df	F	p-value	Signif.	R-sq.(adj)
Scarabaeidae otu11	*N.* cf. *hispida*	1	1	22.27	5.36e-06	***	0.225
	N. thebaica/gambiensis	1	1	21.74	6.77e-06	***	
Scarabaeidae otu33	*N.* cf. *hispida*	1.651	1.878	11.56	3.1e-05	***	0.261
	N. thebaica/gambiensis	1.423	1.667	11.92	0.0014	**	
Scarabaeidae otu15	*N.* cf. *hispida*	1.000	1.000	0.031	0.860		-0.0087
	N. thebaica/gambiensis	1.638	1.87	0.410	0.689		
Ectobiidae otu4	*N.* cf. *hispida*	1.909	2.260	2.765	0.0634		0.0806
	N. thebaica/gambiensis	1.867	1.982	0.111	0.9032		
Macrotermes bellicosus	*N.* cf. *hispida*	2.773	2.961	1.446	0.253		0.0324
	N. thebaica/gambiensis	1.757	1.941	0.241	0.754		
Calycopis sp. *otu13*	*N.* cf. *hispida*	2.937	3.451	3.907	0.00884	**	0.0858
	N. thebaica/gambiensis	1.000	1.000	0.052	0.81975		
Notodontidae otu69	*N.* cf. *hispida*	1	1	0.694	0.406		-0.00762
	N. thebaica/gambiensis	1	1	0.153	0.696		
Gryllidae otu62	*N.* cf. *hispida*	2.039	2.400	2.356	0.101		0.0511
	N. thebaica/gambiensis	1.863	1.981	0.481	0.636		
Trichonephila fenestrata	*N.* cf. *hispida*	1.815	2.165	2.308	0.123		0.191
	N. thebaica/gambiensis	1.000	1.000	0.000	0.990		
Araneae otu61	*N.* cf. *hispida*	1	1	5.152	0.0245	*	0.0564
	N. thebaica/gambiensis	1	1	0.453	0.5016		

3.3. Seasonal prey tracking

The detailed analysis of the seasonal dynamics in the consumption of the 10 most frequent prey OTUs, along with seven additional OTUs representing key crop pests, revealed distinct temporal trends in prey occurrence throughout the sampling period (Figure 3.4).

For *Nycteris* cf. *hispida*, the agricultural pest *Pseudacanthotermes* sp. otu41 peaked in March, followed by an increase in the consumption of Gryllidae otu62 in May. In June, *Calycopis* sp. otu13 reached its highest occurrence, coinciding with elevated values of Scarabaeidae otu11 and otu33, all of which subsequently decreased the following month. In July the crop pests *S. littoralis*, *Achaea ezea* and *Trinervitermes togoensis* had their highest consumption. From September onwards, *Trichonephila fenestrata* and Araneae otu61 became more prevalent, with a high resurgence of Scarabaeidae otu11 and otu33. Scarabaeidae otu15, Ectobidae otu4, Notodontidae otu69 and the crop pests *Macrotermes bellicosus*, *Orseolia oryzae* and *Anarsia* sp. otu860 showed relatively stable consumption patterns throughout all the sampling periods.

In contrast, the wPOO profiles of *Nycteris thebaica/gambiensis*, sampled only from September onwards, showed more constrained seasonal trends. A notable peak was observed in November for Scarabaeidae otu11 and otu33, along with *Ruspolia* sp. otu81, although values for the latter two declined in December. In December, there was a marked increase in the consumption of Notodontidae otu69, becoming one of the dominant prey items.

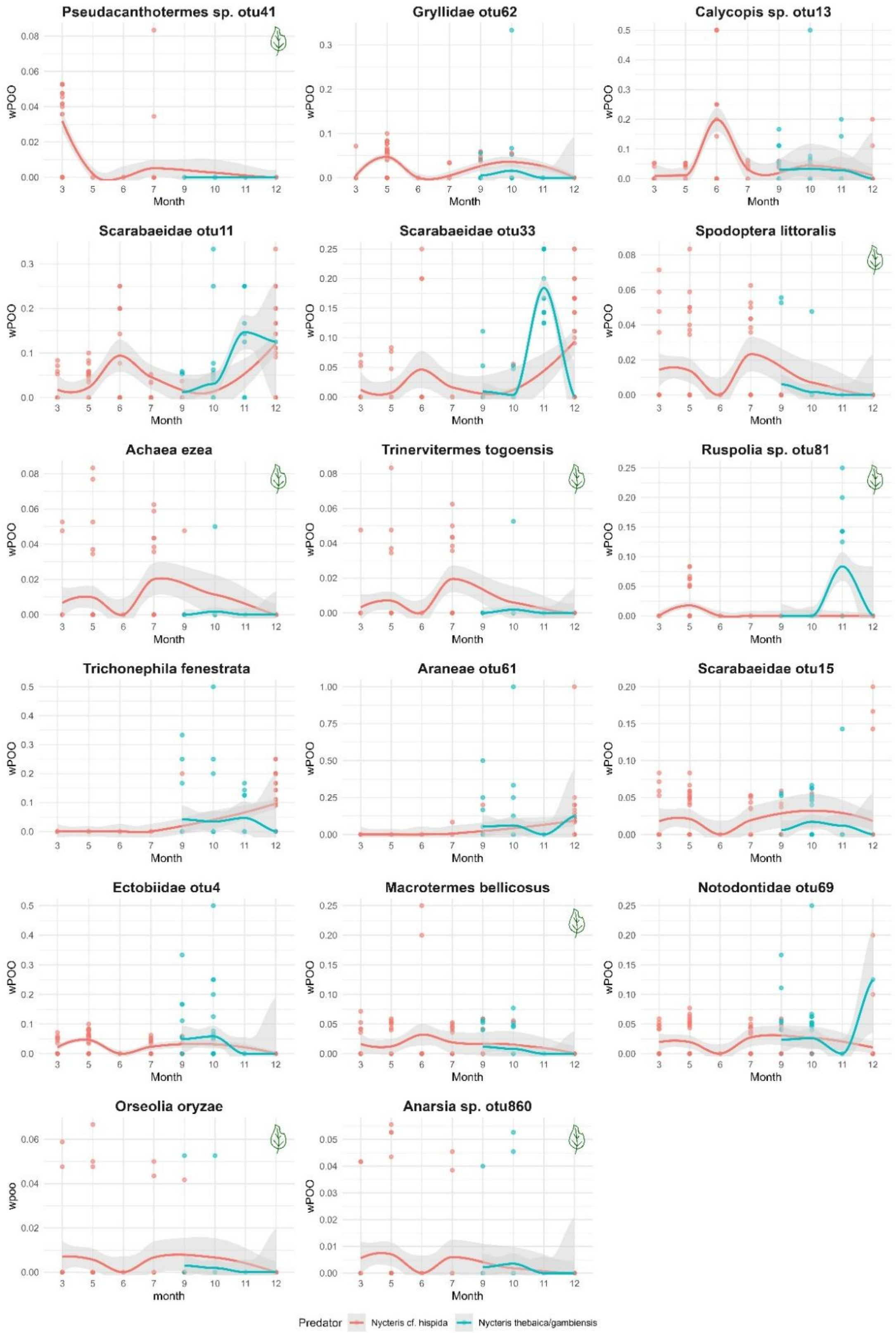

Figure 3.4. Observed variation in the wPOO of 17 prey OTUs, including the 10 most frequent in the diet of both predators and eight key pests (leaf symbol). *M. bellicosus* is among the most frequent and is also classified as a key pest. The dots represent the observed wPOO values and the shaded areas represent a 95% confidence interval. The trend was estimated using LOESS smoothing (span=0.6). Note that the scale of the Y-axis differs between the graphs.

4. Discussion

In this study, we used DNA metabarcoding to analyse the diet of two Nycteridae species roosting in agricultural fields in West Africa. Taxonomic and systematic uncertainties in African bats, especially within *Nycteris*, combined with incomplete molecular reference databases, particularly for invertebrates, pose major challenges for accurately identifying both predator species and their prey. Despite these challenges, we confirmed that both bat species exhibited distinct dietary dynamics and temporal shifts in prey consumption. Few studies have assessed the potential of *Nycteris* species for crop pest suppression and, here, we show that their diet includes known agricultural pests such as *S. littoralis*, *A. ezea*, *T. togoensis* and *M. bellicosus.*

4.1. Spatial and temporal overlap in the diet of *N.* cf. *hispida*

Temporal variation emerged as a key factor in the dietary composition of *Nycteris* cf. *hispida*, likely reflecting seasonal fluctuations in prey occurrence, as May represents a transitional period toward the rainy season and December the beginning of the dry season. Arthropod populations often exhibit marked seasonal variation in abundance and composition (Mavasa et al., 2022; Wolda & Wong, 1988) and the variation in dietary composition of *N.* cf. *hispida* appears to align with these seasonal transitions. This is further evidenced by the significant association of certain prey taxa with specific months. For instance, moths (Lepidoptera) were more frequently preyed upon in May, aligning with reports of increased moth abundance during the transition from dry to wet seasons in the lowland rainforests of Mount Cameroon (Maicher et al., 2018). Orthoptera also showed a stronger association with this period, possibly reflecting early responses to pre-rainy season conditions such as rising humidity, which can stimulate their activity (Omotosho & Abiodun, 2007) and has similarly been described in Central America (Panama) (Richards & Windsor, 2007).In addition, bat reproductive phenology may also influence dietary shifts. The presence of offspring in roost A during May suggests that a proportion of females were pregnant or lactating. Similar observations have been reported in the Garamba National Park, Democratic Republic of Congo, where births occurred in April with lactation continuing until the end of July (Kingdon, 2013). Given that both pregnancy and lactation substantially increase energetic demands in female bats, this may explain the higher consumption of energy-rich prey such as Lepidoptera during this period (Verkerk et al., 2007).

Roost location had a significant, though weaker, influence on the diet composition of *N.* cf. *hispida*. Given the energetic costs of travel (MacArthur & Pianka, 1966), bats are expected to favour nearby, high-quality foraging patches to maximise energy intake. As a gleaner with a marked preference for wooded habitats such as forests and wooded savannas (Kingdon, 2013), *N.* cf. *hispida* likely foraged primarily in the dominant woodland surrounding both roosts. This habitat likely offered similar prey resources, particularly understory and ground-dwelling arthropods, such as scarab beetles (Scarabaeidae otu33), orb-weaving and other spiders (*N. penicillipes*, Araneidae otu347) and cockroaches (Ectobiidae otu4) (Gonçalves et al., 2021; Kwok & Eldridge, 2015; Nogueira & Pinto-da-Rocha, 2016). However, differences in the spatial arrangement and proximity to other habitats, such as rice fields or cashew orchards, likely influenced fine-scale foraging patterns by providing alternative or supplementary resources. The foraging range of *N.* cf. *hispida* remains poorly known and further research on its spatial ecology is needed to assess how microhabitat variation shapes its diet.

Other factors such as prey availability, age of individual bats, landscape structure and weather, may also influence dietary patterns (O'Rourke et al., 2022). Future studies should implement a sampling strategy that encompasses diverse populations and spans multiple seasons to better capture the influence of these variables.

4.2. Annual variation in prey consumption

The two *Nycteris* species consumed a diverse array of arthropod prey across multiple orders and functional groups, with notable seasonal and species-specific variation. Dietary differences between the two bats align with previous reports describing *Nycteris hispida* as a coleopteran consumer (Monadjem et al., 2010), while lepidopterans are considered a primary prey for *Nycteris thebaica* (Kingdon, 2013; Taylor et al., 2017).

These differences, alongside variations in overall prey composition and seasonal consumption, suggest some degree of niche differentiation, a known mechanism for reducing competition among sympatric insectivorous bats (Dammhahn et al., 2015; Jiang et al., 2008). The concept of ecological niches postulates that co-occurring species must partition resources along at least one dimension of their niche, such as diet, habitat or temporal activity, to coexist and avoid competition (Hardin, 1960; Schoener, 1974). Several mechanisms could lead to such separation, including morphological traits, sensory capabilities, divergence in prey selection, variation in foraging style, time and differences in habitat use (Beilke et al., 2021; Starik et al., 2021).

While both species can glean and slow hawk, *N. thebaica* is also able to forage by fly-catching (Kingdon, 2013), a behaviour that allows it to capture fast-flying prey in open spaces. This greater foraging versatility may allow *N. thebaica/gambiensis* to exploit aerial prey like Lepidoptera more effectively than *N.* cf. *hispida*, which appears to rely more on ground-dwelling or surface-associated prey such as Coleoptera (Schnitzler & Kalko, 2001). Such behavioural differences likely contribute to microhabitat partitioning, with *N.* cf. *hispida* favouring cluttered vegetation and *N. thebaica/gambiensis* frequenting more open areas, minimising the direct competition for food resources (Andriollo et al., 2021).

Morphological differences may further support this niche differentiation. Although both bats are morphologically similar, *Nycteris* cf. *hispida* is smaller, with a lower aspect ratio and low wing loading *(N. hispida*: aspect ratio = 4.8, wing loading = 5.4, N.m^{-2}; *N. thebaica*: aspect ratio = 6.7, N.m^{-2}, wing loading=5.3; Monadjem et al., 2010), suggesting a greater manoeuvrability in dense habitats. In contrast, the morphology of *N. thebaica/gambiensis*, including longer wings and greater flight efficiency, may be more suited for faster aerial foraging and fly-catching in open spaces (Heim et al., 2021; Norberg & Rayner, 1987). Although little is known about *N. gambiensis,* its morphological similarity to *N. thebaica* (Kingdon, 2013) suggests it may share similar adaptations for aerial foraging and fly-catching in more open microhabitats.

Temporal variations in prey consumption were more pronounced in *Nycteris* cf. *hispida*, which exhibited significant temporal shifts in multiple prey taxa across the sampled period. This pattern was likely influenced by differences in sampling coverage: *N.* cf. *hispida* was sampled over a broader range of months, increasing the likelihood of capturing seasonal dietary shifts. In contrast, *N. thebaica/gambiensis* showed significant temporal variation in fewer prey categories. While this might suggest lower dietary flexibility or a more stable prey base, it more likely reflects the limited sampling, which was restricted to September-December. Notably, *N. thebaica* has been previously described as a seasonally adaptable forager (Bowie et al., 1999; Kingdon, 2013), indicating that dietary plasticity is expected in this species. The absence of data from part of the year means that key seasonal transitions, such as the shift from the dry to the rainy season, may have been overlooked for this species. As a result, potential seasonal changes in diet might not have been detected, underestimating the degree of dietary plasticity in *N. thebaica/gambiensis*. This sampling limitation should be accounted when interpreting the differences in seasonal patterns between the two species.

Nevertheless, the recorded differences may represent subtle niche differentiation, reducing competition during periods of co-occurrence in the same roost. Similar patterns of dietary partitioning and seasonal variation have been reported in other sympatric bat species, where differences in diet breadth were linked to foraging strategies and habitat use (Starik et al., 2021). To better understand whether such niche partitioning occurs consistently between *Nycteris* cf. *hispida* and *N. thebaica/gambiensis*, further sampling across the full annual cycle is needed to capture potential seasonal shifts and confirm the observed patterns of dietary flexibility.

4.3. Seasonal prey tracking

Both bat species exhibited fluctuations in prey consumption, demonstrating their dietary plasticity and suggesting that they track seasonal changes in arthropod occurrence, including agricultural pests.

In *Nycteris* cf. *hispida*, seasonal variation in the diet and distinct temporal peaks in the consumption of diverse taxa may suggest an adjustment to prey occurrence and we can speculate that prey consumption coincides with periods of peak arthropod activity. This opportunistic foraging strategy allows *N.* cf. *hispida* to prioritise readily available prey, maximising energy intake while thriving in dynamic environments such as agroecosystems (Symondson et al., 2002). For instance, the consumption of crop pests such as *S. littoralis*, *A. ezea* and *T. togoensis* was highest during the rainy season and the rice-seeding, as well growing period of other crops (FAO, 2024). These are times when insect emergence and pest outbreaks tend to be most intense (Denlinger, 1980) . Similar patterns have been observed in other insectivorous bats, where prey selection aligns with seasonal availability rather than occurring randomly (Clare et al., 2011; Tiede et al., 2020), reflecting a high dietary adaptability.

In contrast, *N. thebaica/gambiensis* exhibited a more restricted pattern of prey consumption, consistent with the shorter sampling period. Nonetheless, distinct prey peaks suggest possible tracking of seasonal prey occurrence, in line with literature describing *N. thebaica* as showing seasonal feeding patterns (Taylor et al., 2011). However, due to the restricted sampling period, we need a more extensive sampling effort covering a full annual cycle to confirm this.

Several pests, such as *M. bellicosus*, *O. oryzae* and *Anarsi*a sp., were consistently consumed throughout the sampled months. This likely reflects their continuous occurrence in the landscape and supports a generalist, opportunistic foraging strategy where bats exploit both consistently present prey and seasonally frequent taxa.

By exploiting both seasonally frequent and consistently frequent pest taxa, these bats may deliver continuous pest suppression services across multiple crop phases. This aligns with findings that insectivorous bats are efficient responders to prey dynamics (Maine & Boyles, 2015a), underscoring their functional importance in agroecosystem resilience and the need to prioritise their conservation in agricultural landscapes.

However, to fully understand the extent of this contribution, future studies should go beyond dietary data and include direct measurements of prey abundance, foraging range, as well as observations or monitoring of bat activity. This would help to confirm whether the observed dietary patterns truly reflect active prey tracking and better quantify the role of insectivorous bats as ecosystem service providers in fragmented agricultural landscapes.

4.4. Consequences for conservation

Our study provides important insights into the general feeding behaviour of *Nycteris* bats in a rice-growing landscape in West Africa, revealing them as adaptable predators capable of exploiting diverse prey in a dynamic environment. Our results support their generalist and opportunistic feeding strategies, including the consumption of arthropods commonly associated with agricultural pests. These findings align with our hypotheses and highlight the potential role of these bats in arthropod control, contributing valuable ecosystem services such as pest regulation in agricultural ecosystems.

Implementing conservation and management strategies that support bat populations in agricultural landscapes is crucial for promoting natural pest control, which in turn can lead to sustainable increases in crop yield and productivity (Tuneu-Corral et al., 2024). These strategies may include enhancing landscape heterogeneity (Kelly et al., 2016), increasing the availability of artificial roosting habitat (Flaquer et al., 2006), managing agrochemical usage (Oliveira et al., 2020) and maintaining water sources (Stahlschmidt et al., 2012). These measures can lead to more diverse, abundant and active bat communities, ultimately reinforcing their ecological role as natural pest suppressors and contributing to more resilient and productive agroecosystems (Puig-Montserrat et al., 2020).

5. References

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Supplementary Materials

Table S1. COI sequences used for taxonomic identification of the two *Nycteris* bat species and selected prey taxa included in dietary graphs. Sequence lengths are shown in base pairs (bp).

OTU ID	COI sequence	Bp
Nycteris cf. *hispida*	TACTCTATACCTACTATTTGGTGCGTGAGCCGGAATAGTAGGAACCGCCCTGAGCCTTTTAATTCGCGCCGAACTAGGACAGCCCGGAGCTCTTTTAGGAGACGATCAAATCTACAATGTTATTGTCACCGCCCATGCTTTTGTAATAATCTTCTTTATGGTTATGCCCATTATGATT	178
Nycteris thebaica/gambiensis	CACTCTTTACTTGCTATTTGGCGCATGAGCAGGAATAGTAGGAACCGCCCTAAGTCTACTAATTCGCGCTGAACTTGGCCAACCTGGAGCCCTCTTAGGAGACGATCAGATTTATAACGTAATTGTAACAGCCCACGCTTTCGTAATAATTTTCTTTATAGTTATGCCCATCATGATC	178
Achaea ezea	ACTTTCATCTAATATTGCACATAGAGGAAGATCTGTAGATCTAGCTATTTTTTCTTTACATTTAGCAGGAATTTCATCTATTTTAGGAGCTATTAATTTTATTACAACAATTATTAATATACGATTAAATAATCTAATATTTGATCAAATACCTTTATTTGTTTGAGCTGTAGGAATTACAGCATTCCTTCTTCTTTTATCTTTA	205
Anarsia sp. otu860	TCTTTCTTCTAATATCGCTCATAGTGGAAGTTCAGTTGATTTAGCTATTTTTTCTTTACATTTAGCTGGAATTTCTTCAATTTTAGGAGCAATTAATTTTATTACAACTATTATTAATATACGGATTAATGGTTTATCATTTGATCAAATACCATTATTTGTTTGAGCCGTAGGTATTACAGCTTTATTATTACTTTTATCTTTA	205
Araneae otu61	ATTGGCTGGATTAGAGGGGCATGCCGGTAGATCTGTAGATTTTGCTATTTTTTCTTTGCATTTAGCAGGGGCTTCGTCAATTATAGGGGCTATTAATTTTATTTCAACAATTATTAATATACGATTTTATGGTATAACCATGGAGAAAGTGCCTTTATTTGTTTGGTCAGTTTTAATTACAGCGGTATTGTTATTATTGTCTTTG	205
Calycopis sp. *otu13*	ATTATCAGGAATACAATCACACAGTGGACCAAGTGTTGATTTAGCAATTTTTGGTTTACACTTAAGTGGTATTAGTAGTATGTTAGGAGCTATGAACTTCATTACAACTCTTTTAAATATGAGAGGTCCAGGTATACGTTTACACAAATTAGCATTATTTGGTTGAGCTGTAGTAATAACAGCAGTATTGTTATTATTATCATTA	205
Ectobiidae otu4	ATTAGCCAGAGGCATTGCTCACGCCGGTGCTTCAGTTGATTTAGCTATTTTTTCTTTACACTTAGCTGGTGTATCATCAATTTTAGGAGCTGTAAATTTTATTTCAACAATTATTAATATGAAACCAATTAGAATAACACCCGAACGAATCCCACTTTTTGTTTGATCAGTGGGAATTACAGCTTTATTACTCTTATTATCACTT	205
Gryllidae otu62	TTATCTACAGGTATTGCTCATGCAGGGGCATCAGTTGATTTAGCAATTTTCTCATTACACTTAGCAGGGATTTCTTCAATTTTAGGAGCAGTAAATTTTATCACAACCATAATTAATATACGAGCACCTGGAATATCTCTAGATCAAACACCACTATTTGTGTGAGCCGTTGGTATTACTGCCCTTCTATTATTATTATCTTTG	205
Macrotermes bellicosus	CCTAGCAAGAGGTATTGCACATGCTGGGGCATCTGTAGATCTTGCCATCTTTTCCCTACACCTAGCGGGAGTATCATCTATCCTAGGAGCAGTAAACTTTATCTCAACAACTATCAACATAAAGCCAAGAAACATAAAACCAGAGCGAATTCCCCTATTCGTATGATCAGTAGCCATTACAGCCCTTCTCCTGTTATTATCACTA	205
Notodontidae otu69	TTTATCATCTAATATCGCACATGGGGGAAGTTCAGTGGACTTAGCTATTTTCTCATTACATTTAGCTGGAATTTCATCAATTCTAGGAGCTATTAATTTTATTA	205

	CAACAATTATTAATATACGATTAAATAGAATAATATTTGATCAATTACCATTATTTGTGTGAGCAGTTGGGATTACTGCATTCTTATTACTACTTTCATTA	
Orseolia oryzae	TCTTTCTTCTATTATTGCTCATACTGGATCTTCAGTAGATTTTTCAATTTTTTCTCTTCATATTGCAGGAATTTCTTCTATTTTAGGAGCTATCAATTTTATTTCAACTATATTAAATATAAAAATTAAATTCTTAAAATTTGATCAAATTTCATTATTTATTTGATCAATTTTAATTACTACAATTTTATTATTATTATCTTTA	205
Pseudacanthotermes sp. otu41	TCTAGCTAGAGGAATTGCACACGCTGGAGCATCTGTAGACTTAGCGATCTTCTCACTACACCTTGCAGGAGTATCATCCATCCTTGGAGCAGTAAACTTCATTTCAACAACTATCAACATAAAACCAAAAAACATAAAGCCCGAACGAATCCCACTATTTGTATGATCAGTTGCTATCACAGCACTACTCCTATTGCTATCACTA	205
Ruspolia sp. otu81	GCTTTCTGCAGGAATTGCTCATGCAGGAGCCTCAGTTGATCTAGCAATTTTCTCCTTACACCTAGCTGGAGTTTCTTCAATTCTTGGGGCTGTAAATTTCATTACTACAACTATTAACATACGAGCCCCAGGTATATCACTAGATCAAACACCTTTATTTGTTTGAGCTGTTGCCATTACGGCCCTCTTGTTACTTCTCTCCCTC	205
Scarabaeidae otu11	ACTATCAGGTATACAAAGCCACAGTGGTCCTAGTGTAGATTTAGCTATATTCGCTCTTCACCTATCAGGGATAAGTAGTCTATTAGGAGCAATGAATTTTATAACAACTATCTTAAACATGAGAAGTCCAGGAATAAGACTACACAAATTGGCTTTATTTGGATGAGCAGTCGTGGTTACAGCAGTATTATTACTTTTATCTTTA	205
Scarabaeidae otu15	ATTATCAGGTATACAAAGTCACAGTGGTCCTAGTGTAGATTTAGCTATCTTCGCTCTTCACCTATCAGGGATAAGTAGTCTATTAGGAGCAATGAATTTTATAACAACTATCTTAAACATGAGAAGTCCAGGAATAAGACTACACAAATTGGCTTTATTTGGATGAGCAGTTGTGGTTACAGCAGTTTTATTACTTTTATCTTTA	205
Scarabaeidae otu33	ATTATCAGGTATACAAAGTCACAGTGGTCCTAGTGTAGACTTAGCAATTTTTGCTCTTCACCTATCAGGGATAAGTAGTCTATTAGGAGCAATGAATTTTATTACAACTATCCTAAACATGAGAAGTCCAGGAATAAGATTACACAAATTGGCTTTATTTGGATGAGCAGTAGTTGTAACAGCAGTTTTATTACTTTTATCTTTA	205
Spodoptera littoralis	CCTCTCCTCTAATATTGCTCATGGTGGAAGATCAGTAGACTTAGCTATTTTTTCCCTTCATTTAGCAGGAATTTCATCTATTTTAGGAGCTATTAATTTTATTACTACTATTATTAATATACGATTAAATAATTTATCATTTGATCAAATACCTTTATTTGTTTGAGCTGTAGGAATTACCGCATTTTTATTATTATTATCTTTA	205
Trichonephila fenestrata	CCTCGCTTCTCTAGATGGGCATGCTGGAAGATCTGTAGATTTTGCTATTTTTTCTTTGCATTTGGCGGGAGCATCTTCAATTATAGGTGCAATTAATTTTATTTCTACAATTATAAATATACGGTCTTTTGGGATAACAATAGAGAAAGTCCCTTTATTTGTTTGATCAGTATTGATTACTGCTATTTTATTATTATTATCTTTA	205
Trinervitermes togoensis	TCTTGCAAGAGGAATTGCCCATGCAGGAGCATCTGTAGACCTAGCAATCTTCTCACTACATCTAGCAGGAGTCTCATCTATCTTAGGGGCAGTAAACTTCATTACAACAACAATTAATATAAAGCCAAAAAGTATAAAACCAGAACGAATTCCCCTATTCGTATGATCAATTGCAATTACCGCCTTGTTATTATTGCTTTCTCTA	205

Table S2. Summary table of crop pests and potential crop pests found in the diet.

Prey	**Ecology**	**Reference**
Achaea ezea	Crop pest	Hargreaves. 1936
Anarsia sp. otu860	Crop pest	Soumya et al., 2017
Macrotermes bellicosus	Rice pest Crop pest	Hill, 2008
Orseolia oryzae	Rice Pest	Edde, 2022; Heinrichs & Barrion, 2004
Pseudacanthotermes sp.	Rice pest Crop pest	Ackonor, 1997; Akpesse et al., 2008
Ruspolia sp.	Rice pest Crop pest	Heinrichs & Barrion, 2004
Spodoptera littoralis	Rice pest Crop pest	Heinrichs & Barrion, 2004; Hill, 2008
Trinervitermes togoensis	Crop pest	Schyra & Korb, 2019

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Table S3. PERMANOVA testing for differences in diet composition of *N.* cf. *hispida* - roost site (Roost), month (Month) or interaction between the two (Roost: Month). The results significance is shown as: *** < 0.001; ** < 0.01.

	Df	SumOfSqs	R^2	F	Pr(>F)	
Roost	1	0.9065	0.04079	3.0362	0.002	**
Month	1	4.5543	0.20496	15.2549	0.001	***
Roost: Month	1	0.9371	0.04217	3.1388	0.003	**
Residual	53	15.8230	0.71208			
Total	56	22.2208	1.00000			

Table S4. Significant associations (*multipatt* function from the 'indicspecies' package) between prey taxa of *N.* cf. *hispida* and roost-month group and the respective p-value. The results significance is shown as: *** < 0.001; ** < 0.01; * < 0.05. Lighter colours indicate lower significance, and darker colours indicate higher significance.

Taxa	A-May	A-Dec	B-May	B-Dec
Araneidae otu347		0.03*		
Orthoptera otu187		0.04*		
Mocis mayeri	0.001***			
Episparis sp. otu1123	0.001***			
Acrididae otu715	0.002**			
Blattodea otu736	0.013*			
Acrididae otu929	0.045 *			
Cicadellidae otu139	0.045 *			
Psocidae otu2043				0.006**
Pisauridae otu583				0.006**
Gesonia stictigramma				0.037*
Phaneroptera sp. otu2761				0.030*
Orthoptera otu138			0.001***	
Elaeidobius kamerunicus			0.001***	
Autocharis fessalis			0.001***	
Araneae otu434			0.001***	
Erebidae otu118			0.001***	
Melanitis leda			0.001***	
Psocidae otu753			0.001***	
Ruspolia sp. otu825			0.001***	
Neoscona penicillipes			0.003**	
Orthoptera otu1525			0.008**	
Lepidoptera otu1224			0.038*	
Achaea ezea			0.035*	
Trichonephila fenestrata		0.001***		0.001***
Scarabaeidae otu33		0.001***		0.001***
Araneae otu61		0.002**		0.002**
Tettigoniidae otu97		0.002**		0.002**
Ectobiidae otu4	0.001***		0.001***	
Gryllidae otu62	0.001***		0.001***	
Tenosius sp. otu505	0.002**		0.005**	
Elaeidobius pilimargo	0.018*		0.021*	
Ruspolia sp. otu81	0.018*		0.021*	
Spodoptera littoralis	0.024*		0.028*	
Coleoptera otu268	0.039 *		0.039 *	
Macrotermes bellicosus	0.042 *		0.042 *	
Calycopis sp. otu13			0.004 **	0.004**
Scarabaeidae otu11	0.002**	0.002**		0.002**

Table S5. Parametric coefficients from the GAM model assessing predation by both *Nycteris* species on different arthropod orders. Significance levels are shown as: *** < 0.001; ** < 0.01; * < 0.05.

Parametric coefficients						
		Estimate	Std.error	t-value	Pr(>|t|)	Signif.
Araneae	(Intercept)	-2.0038	0.1320	-15.175	<2e-16	***
	N. thebaica/gambiensis	0.2058	0.4306	0.478	0.633	
Blattodea	(Intercept)	-2.4157	0.1481	-16.306	<2e-16	***
	N. thebaica/gambiensis	-4.3669	4.6308	-0.943	0.347	
Coleoptera	(Intercept)	-1.76043	0.07286	-24.16	< 2e-16	***
	N. thebaica/gambiensis	-2.38557	0.35186	-6.78	2.15e-10	***
Diptera	(Intercept)	-2.8483	0.1875	-15.187	<2e-16	***
	N. thebaica/gambiensis	-0.7086	0.7056	-1.004	0.317	
Hemiptera	(Intercept)	-2.1891	0.1106	-19.786	<2e-16	***
	N. thebaica/gambiensis	-5.3891	4.3420	-1.241	0.216	
Hymenoptera	(Intercept)	-2.9875	0.1853	-16.120	<2e-16	***
	N. thebaica/gambiensis	-10.1647	51.7514	-0.196	0.845	
Lepidoptera	(Intercept)	-1.79017	0.08539	-20.964	< 2e-16	***
	N. thebaica/gambiensis	0.82728	0.38237	2.164	0.032	*
Orthoptera	(Intercept)	-1.9848	0.1190	-16.672	<2e-16	***
	N. thebaica/gambiensis	0.2599	0.2678	0.971	0.333	
Psocodea	(Intercept)	-3.1474	0.1713	-18.371	<2e-16	***
	N. thebaica/gambiensis	0.5439	1.0024	0.543	0.588	

Table S6. Parametric coefficients from the GAM model assessing predation by both *Nycteris* species on different arthropod OTUs. Significance levels are shown as: *** < 0.001; ** < 0.01; * < 0.05.

Parametric coefficients						
		Estimate	Std.error	t-value	Pr(>|t|)	Signif.
Scarabaeidae otu11	(Intercept)	-2.8963	0.1378	-21.013	< 2e-16	***
	N. thebaica/gambiensis	-2.5807	0.6458	-3.996	9.77e-05	***
Scarabaeidae otu33	(Intercept)	-3.6111	0.2251	-16.042	< 2e-16	***
	N. thebaica/gambiensis	-7.0069	6.1543	-1.139	0.257	
Scarabaeidae otu15	(Intercept)	-4.0761	0.1992	-20.459	<2e-16	***
	N. thebaica/gambiensis	-4.2546	4.9900	-0.853	0.395	
Ectobiidae otu4	(Intercept)	-4.4925	0.3945	-11.387	< 2e-16	***
	N. thebaica/gambiensis	-17.7397	57.6606	-0.308	0.759	
Macrotermes bellicosus	(Intercept)	-4.8339	0.5305	-9.111	3.28e-16	***
	N. thebaica/gambiensis	-8.8491	27.1935	-0.325	0.745	
Calycopis sp. *otu13*	(Intercept)	-3.7011	0.3255	-11.371	<2e-16	***
	N. thebaica/gambiensis	0.3126	0.7935	0.394	0.694	
Notodontidae otu69	(Intercept)	-4.2402	0.2357	-17.992	<2e-16	***
	N. thebaica/gambiensis	0.5981	0.6514	0.918	0.36	
Gryllidae otu62	(Intercept)	-4.5741	0.3878	-11.795	<2e-16	***
	N. thebaica/gambiensis	-20.1300	64.7249	-0.311	0.756	

Trichonephila fenestrata	(Intercept)	-5.939	2.012	-2.952	0.00363	**
	N. thebaica/gambiensis	2.599	2.148	1.210	0.22807	
Araneae otu61	(Intercept)	-4.756	1.071	-4.440	1.66e-05	***
	N. thebaica/gambiensis	2.364	1.423	1.661	0.0986	