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Dinner Time: Evidence for Temporal and Dietary Niche Partitioning in Insectivorous Bats in Cameroon

Analysis Project - Data

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CONTENTS

Acknowledgments, page 3

Abstract, page 4

Introduction, page 5

- **i.** Species Coexistence
- **ii.** Coexistence in Bats
- **iii.** Challenges in Studying Bats
- **iv.** Conservation Implications
- **v.** Study Site Background
- **vi.** Objectives

Methods and Materials, page 10

- **i.** Study Area
- **ii.** Bat Sampling
- **iii.** Statistical Methods
- **iv.** Activity/Temporal Overlap
- **v.** DNA Extraction and Metabarcoding
- **vi.** Diet Richness and Diversity
- **vii.** Diet Overlap
- **viii.** Incidence Frequency of Insect Orders
- **ix.** Ethics and License Statement

Results, page 13

- **i.** Temporal Niche
- **ii.** Dietary Studies
- **iii.** Diet Composition
- **iv.** Diversity and Richness
- **v.** Dietary Overlap
- **vi.** Frequency Incidence of Prey

Discussion, page 20

- **i.** Temporal Niche
- **ii.** Dietary Niche Breadth
- **iii.** Niche Overlap (perMANOVA, NMDS, Pianka, Db-RDA)
- **iv.** Frequency of Occurrence
- **v.** Limitations

Conclusion, page 23

References, page 25

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ABSTRACT

Bats account for 20% of mammal species, forming highly diverse communities in most terrestrial ecosystems. Yet, for all this diversity, their morphology and functional traits vary very little between species. This poses an interesting question for coexistence theory: How are so many similar species able to coexist?

I tested for evidence of temporal and dietary niche partitioning in two genera of insectivorous bats in Cameroon, including a newly identified Cryptic species. I found evidence for temporal and dietary segregation of the Cryptic population from closely related species, prompting further research into how these mechanisms contribute to the emergence and coexistence of cryptic species in bat communities.

Furthermore, I found the diet composition varies more between genera than it does within them, supporting the widely held assumption that phylogenetic relatedness implies smaller fitness differences between species owing to a shared niche history.

INTRODUCTION

SPECIES COEXISTENCE

Coexistence describes the persistence of multiple distinct populations within a community through time without one outcompeting another to extinction (Chesson, 2000a) For this to be possible, the density of each group must be more strongly impacted by competition within their group, than by competition with other groups (Chesson, 2000a). Understanding the forces at play in real-life ecosystems allowing species to coexist have been a central theme in ecology over the past century (Letten et al., 2017; Siepielski & McPeek, 2010).

Coexistence theory (Chesson, 2000) proposes two broad categories of mechanisms that facilitate coexistence: stabilising and equalising mechanisms. Equalising mechanisms reduce the fitness differences between groups, reducing the impact of any competitive interactions between groups. Stabilising mechanisms promote differences between species, thus reducing the number of competitive interactions with other groups (Chesson, 2000a). In both cases, the result is a reduction in net competition, or impact upon population size, between different groups.

Stabilising mechanisms include the various axes upon which species specialise, through resource use, life-history, density distribution through a landscape, or predator and natural enemy susceptibility. Stabilising mechanisms are negatively density dependent, with growing populations having increasingly small average fitness as resources become more limited and natural enemy densities increase (Chesson, 2000a; Letten et al., 2017).

Equalising mechanisms reduce the fitness differences between species. This reduces the effect size that species have on other similar species, as they are less likely to out-compete another equally matched group (Letten et al., 2017). As described in Hubbell's neutral theory of biodiversity and biogeography (Hubbell, 2001) – essentially a null comparison to stabilising mechanisms – if species are equal in their per capita birth, death, speciation and dispersal rates, then they should be able to coexist within an ecological community without outcompeting one another.

In practice, understanding how – and if – species coexist stably within real-world ecological communities is challenging. Competition can occur over long ecological time frames, meaning simply observing species co-occurring within a community is not robust evidence that one will not ultimately outcompete the other (Siepielski & McPeek, 2010). The acid test to demonstrate that two groups are in stable coexistence would be to totally remove one group, and later re-introduce it. If the reintroduced group can re-colonise and reach its previous density, then the two species are coexisting stably. Such studies are rarely practical or ethical in real-world systems. This does not make the study of coexistence redundant, however.

Even under a 'null' model in which species are constantly either being outcompeted, or outcompeting other co-occurring species, Chesson's stabilising and equalising mechanisms still provide explanations for why the rate at which species out-compete each other - and hence the richness supported by a community - can very between communities so drastically (Chesson, 2000b, 2000a)

COEXISTENCE IN BATS

Chiroptera are an incredibly species rich order, accounting for around 1 in 5 mammal species (Teeling et al., 2005) .The only mammal order comprising more species are rodents (Rodentia). Unlike Rodentia, morphology varies very little across Chiroptera, likely driven by the constraints imposed by flight (Barclay & Brigham, 1991; Norberg & Rayner, 1987). Yet, Chirpotera co-occur at very high species densities in a range of ecosystems ((Clare et al., 2011) and cryptic species are frequently identified (Hulva & Horáček, 2006; Jacobs et al., 2006; Jones & Van Parijs, 1993; Mayer & Von Helversen, 2001; Novella-Fernandez et al., 2020; Roswag et al., 2019; Thoisy et al., 2014). The insectivore guild of Chiroptera appear to be even further constrained than other bats in their morphology: the major features of the ecology of all insectivores are the same; nocturnal, flying predators with small body size, foraging using echolocation to navigate their environment(Barclay & Brigham, 1991; Kingston et al., 2000; Saunders & Barclay, 1992). Despite of this, bat community assemblages are often incredibly diverse in tropical forests , even sharing roost space in mixed colonies (Furey & Racey, 2015).This coexistence must be facilitated either by very small fitness differences between species, or these highly similar species must differ on some axis enough to reduce interspecific competition (Chesson, 2000b).

CHALLENGES IN STUDYING BATS

Bats provide important services such as mosquito and agricultural pest suppression, and ecosystem functions such as herbivore suppression in regenerating forests ((Kalka & Kalko, 2006; Kunz et al., 2011)). They exist at high densities in many at-risk habitats, such as tropical rainforests (Clare et al., 2011; Gallery, 2014; Kalka & Kalko, 2006; Nkrumah et al., 2016). Understanding the mechanisms facilitating their coexistence would likely be valuable in both efforts to retain these services, to communicate their value to key stakeholders, and to mitigate against species loss in those systems where their habitat is at most risk(Rice & Greenberg, 2000). Thus, the lack of study into bat coexistence is evidently not for lack of motivation – but may be limited by the practicality of studying bat coexistence.

Of all the axis along which species can segregate, diet has by far received the most attention within the animal kingdom. (Adler et al., 2007; Chesson, 2000a; Gallery, 2014; Letten et al., 2017) In bats, three major factors make observational studies on the diet of insectivorous bats impractical. (1) Bats are nocturnal predators, meaning studies can only be carried out in poor light conditions where

observation is a challenge (Barclay & Brigham, 1991); (2) bats handle prey rapidly while foraging, meaning identifying a prey species visually before it is eaten is near impossible(Aldasoro et al., 2019; Barclay & Brigham, 1991; Smout et al., 2010; Vesterinen et al., 2018) ; and (3) coexisting species are very similar morphologically, making visual identification of species in flight unreliable(Aldasoro et al., 2019; Smout et al., 2010).

Historically, analysing bat diets depended on physical identification of prey remains ((Aldasoro et al., 2019). By capturing bats and taking faeces samples, more reliable methods can be used to identify bat species, such as echolocation frequency and forearm length. However, identifying invertebrate species visually from faecal remains offers limited accuracy and is labour intensive for large sample sizes (Aldasoro et al., 2019), and may be limited by presence of undescribed arthropod species(Burgar et al., 2014).

A major innovation in recent years has been the use of DNA metabarcoding to identify the diet composition of species from their faeces. This technique employs high-throughput next generation sequencing to sort prey DNA fragments into groups of closely related individuals, known as operational taxonomic units (OTUs) (Brown et al., 2014).

In bats, this has facilitated an explosion in diet studies (Aizpurua et al., 2018a; Alberdi et al., 2020a, 2020b; Arrizabalaga-Escudero et al., 2018; De Oliveira et al., 2020; Galan et al., 2018; Tournayre et al., 2020). With DNA metabarcoding, diet studies in bats have become far more straightforward, and previously inaccessible hypotheses about bat dietary niches can now be tested. Coexistence studies often assume that fitness differences between insectivorous bat species are likely small owing to close phylogenetic relatedness and an implied shared niche history(Arrizabalaga-Escudero et al., 2018; Godoy et al., 2014). An interesting exception to this assumption are the Rhinolophidae and Hipposideridae families of insectivorous bats. Recent studies have revealed their phylogenetic position as part of the megabat branch and evolutionarily distinct from other insectivores (Amador et al., 2018), - and as such we cannot rely upon phylogenetic relatedness to explain how they coexist within insectivorous bat assemblages.

Furthermore, in tropical rainforests, mutation and evolution rates for mammals are more rapid than in temperate ecosystems (Wright et al., 2006).Basing these assumptions on phylogenetic relatedness naively assumes that fitness differences will not arise between sister taxa over relatively short evolutionary time frames.

Diet niche segregation has been identified in insectivorous bats across Europe (Aldasoro et al., 2019; Roswag et al., 2019) , though distinct diets do not necessarily provide evidence of trophic resource partitioning. Arlettaz et al., (1997) found the diets of two sister bat species in the genus Myotis bats

to be distinct but were no different in sympatry or allopatry, suggesting that their dietary segregation was not driven by interspecific competition(Arlettaz et al., 1997).

Additionally, given insect abundances can vary greatly with habitat features within forests (Saunders & Barclay, 1992), habitat partitioning may be driving dietary segregation, rather than competitive forces. Another study by Roswag *et al* (2019) found the diets of three newly discovered cryptic bats to have greater overlap interspecifically than intraspecifically (Roswag et al., 2019). The dietary features driving species coexistence may occur at a finer scale than many studies test for. Novella Fernandez *et al* (2020) found that where two species in the genus *Myotis* with highly similar diets co-occurred at fine spatial scales, they had significantly lower diet overlap (Novella-Fernandez et al., 2020).

A further potential axis for partitioning is the temporal niche, or the time at which a species is most active during the night. Light conditions, temperature and the activity levels of different insects vary throughout the night in tropical rainforests (Mata et al., 2020) yet to the best of my knowledge, temporal segregation of insectivorous bats has only been studied once in tropical forests (De Oliveira et al., 2020). Partitioning of temporal niches in nocturnal species has seldom been studied, though a handful of studies have found evidence supporting its relevance as a stabilising mechanism. Tropical forest Carnivora in Borneo (Nakabayashi et al., 2021) and insectivorous bats in Costa Rica(De Oliveira et al., 2020) have both been found to segregate their temporal niche. As with habitat partitioning, temporal partitioning might drive the differences in diets identified between populations, yet the effect of time is rarely tested.

CONSERVATION IMPLICATIONS

In West and Central Africa, bat diversity has received little attention. 18.2% of recognised bat species in sub-Saharan Africa are listed as data deficient by the IUCN (IUCN, 2005).The tropical rainforests and Cacao farms of the region are home to high bat species diversity (Carodenuto, 2019; Patterson et al., 2020). Cryptic diversity is high(Patterson et al., 2020), indicating that the true extent of this diversity has yet to be understood in the area. This is a worrying trend for a region at high risk of exploitation in the near future (Carodenuto, 2019; Ordway et al., 2017).

Although currently the largest Cacao producing region in the world, West and Central Africa are considered high risk for future exploitation for oil palm or rubber production (Ordway et al., 2017). Traditional Cacao farming occurs at small scales in mixed agroforests, where an overstory of forest trees are retained above cacao plants. The levels of diversity retained on these farms relative to virgin forest are high compared to other major tropical cash crops such as Oil Palm and Rubber, which are grown in monoculture with a drastic biodiversity cost (Rice & Greenberg, 2000; Ruf, 2011). Conversion of these farms to oil palm or rubber production would likely have devastating impacts on

local diversity. Effectively conserving these ecosystems importantly requires understanding baselines of the biodiversity present. The rate at which cryptic bat species are identified in these regions, and the data-deficient status of so many species on the IUCN red-list demonstrates that further work must be done if bats in these regions are to be effectively baselined.

STUDY SITE BACKGROUND

Biodiversity Initiative have been conducting sampling on bats around Cacao farms in Cameroon since 2017. Within these at-risk systems, generating useful data can directly influence stakeholder behaviour on the ground if handled appropriately. Recent sampling efforts identified the presence of a suspected Cryptic bat within the genus *Hipposideros.* It was labelled as such due to distinctive echolocation frequency characteristics and forearm length when compared to other Hipposideros bats. These differences were statistically significant (Hothersall 2021, Unpublished Data) indicating that the samples did represent a distinct cryptic population. Bat call echolocation frequency has long been used to reliably distinguish between insectivorous bat species (Fenton & Bell, 1981),and dictates the size of prey they can detect (Barclay & Brigham, 1991). Wing morphology, including forearm length, has been found to predict diet (De Oliveira et al., 2020), and individual niche specialisation in insectivorous bats in other communities (Barclay & Brigham, 1991). The identification of this cryptic population poses as to whether these morphological traits will contribute to a distinct dietary niche from co-occurring *Hipposideros* species.

OBJECTIVES

This study tested for evidence of niche partitioning within and between genera in insectivorous bats sampled by Biodiversity Initiative in Cameroon over the 2017 and 2018 field seasons. I compared five abundant study species from the sites: *Hipposideros ruber, Hipposideros caffer, Hipposideros fuliginosus*, the suspected Cryptic *Hipposideros*, and a commonly occurring member of a sister taxon, *Rhinolophus alcyone*. Dietary data from the bats was available from metabarcoding analysis run by Biodiversity analysis. I built a picture of niche partitioning in these species by addressing the following objectives:

1. Do species display different temporal activity patterns?
2. Do species differ in their dietary breadth?
3. Do species diets overlap with each other?
4. Are there fine-scale differences in the dietary composition of different species?

METHODS AND MATERIALS

STUDY AREA

The study was conducted across 14 Cacao farms in Cameroon, Africa. These farms were in three distinct landscapes: Ayos, Bokito and Konye. Konye comprised the largest share with 7 farms, followed by Ayos with 6 farms, and Bokito with 1 farm.

BAT SAMPLING

A total of 28 nights of bat sampling were conducted, using 17-20 ground-level mist nets at each of thr 14 farms, over 1-3 nights. Sampling began at dusk (18:00) and finished at 00:00. The mist nets were inspected at 15-minute intervals in the first 2 hours of night (>50% of captures at this time), and 20-minute intervals thereafter. Bats were identified, measured, and faecal samples were collected for DNA analysis. Species identification followed Rosevear (1965), Hayman & Hill (1971), Patterson & Webala (2012) and Happold *et al* (2013); Taxonomy followed ACR (2019).

STATISTICAL METHODS

I carried out all analyses in R v4.0.3 (R Core Team 2020). I performed model selection using likelihood ratio tests (LRTs) using the lrtest() function in the R package lmtest (Hothorn et al., 2022)to compare nested models. Starting with the most complex plausible model, terms were sequentially removed, and models compared to one another. Non-significant differences between models in the LRTs implied the terms were not important to model fit (log-likelihood) and were sequentially discarded, until the 'best-fit' model was reached.

ACTIVITY / TEMPORAL OVERLAP

To test whether the study species segregate their temporal 'niche', I tested for pairwise differences between species in the time they were captured during sampling using a linear model. Given that mist-net trapping depends upon bats actively flying into the traps, I consider it a robust assumption that capture incidence throughout the night would correlate with actual bat activity.

For the analysis, I created a new continuous variable from the raw capture data, 'Time Difference'. This was calculated as the time surpassed after 18:00, when sampling sessions typically started. These time differences were rounded to the nearest 15 minutes, thus grouping the bats into time bins. A second variable was created for each bat, the 'Proportion' of captures they represented ($1/n_{group}$) in order to plot comparisons of capture density between species.

The best-fit model was assessed using likelihood-ratio tests (LRTs) as described in the statistical methods section. Time difference was log-transformed in order not to violate the assumption of

normality in the linear mixed-effects model. The *lmer()* function in *lme4* (Bates et al., 2015) was used to fit a linear mixed-effects model of log-transformed time difference as the dependent variable and species as the predictor, including farm as a random effect. A post-hoc Tukey test was applied using the *emmeans()* function in the R package *emmeans* (Lenth, 2023) for pairwise comparisons between bat species.

DNA EXTRACTION AND METABARCODING

All DNA extraction and metabarcoding was conducted by Biodiversity Initiative prior to this study. A summary of their methods is provided below:

Faecal samples were preserved in Longmire buffer and DNA extracted, following the protocol described by Mata *et al* (2016). The samples were then amplified using the ZBJ primer ((Zeale et al., 2011), which was designed for metabarcoding the faeces of insectivorous bats. Following successful extraction, the samples were sent for metabarcoding.

The sequenced samples were then clustered into Operational Taxonomic Units (OTUs), which are used to represent approximate species equivalents within the sample. Further bioinformatics analysis was conducted to identify the OTUs to order level vis comparison with reference genomes in GenBank and the Barcode of Life Database (BOLD).

DIET RICHNESS AND DIVERSITY

I compared both dietary richness and prey diversity between species by fitting a linear mixed-effects models using the *lmer()* function in *lme4*, then generated pairwise comparisons with a post-hoc Tukey test using the *pairs()* function in the R package *emmeans().* In order to reach the best-fit models, I used Likelihood-Ratio tests (LRTs) to compare nested models, as is described in the statistical methods section.

I calculated dietary richness for each individual bat as the total number of distinct OTUs present in its diet. I calculated dietary diversity for each sample using Shannon's Diversity Index in the R package *vegan* (Oksanen et al., 2022). Shannon's diversity index has the benefit over richness of considering the abundance of each prey item in diet, estimated using the number of reads. The formula used to calculate Shannon's index is shown below:

$$H = -\sum[(p_i) \times \log(p_i)]$$

H = Shannon diversity index;

$\mathbf{P_i}$ = proportion of individuals of $_i$-th species in a whole community

DIET OVERLAP

I created ordinations to visualise diet overlap between species with nonmetric multidimensional scaling (NMDS) in the R package vegan with the *metaMDS()* function. Three complementary metrics were used to describe differences in dietary niches between study species: 1) distance-based redundancy analysis (db-RDA) and 2) perMANOVA to describe diet dissimilarity; and 3) Pianka's index of niche overlap, to generate pairwise values for niche overlap of species. (1) I calculated db-DRA values at OTU level with 999 permutations to compare Jaccard distances between the diets of individual bats using the *metaMDS()* and *capscale()* functions in the R package vegan. I used the *multiconstrained()* function in the package *BiodiversityR* (Kindt, 2023) to obtain pairwise comparisons between species. (2) I conducted perMANOVA analysis for all pairwise comparisons of the study species using the *paiwise.adonis2*() function in the package *pairwiseAdonis* (Martinez Arbizu, 2017). Farm was included as a stratum in the models to account for variation between sites. (3) I calculated Pianka's index at OTU level for each pairwise species comparison within the sample, using the *niche.overlap.boot()* function in the R package *spaa* (Zhang, 2016). 500 bootstraps were run for each pairwise comparison to generate 95% confidence intervals around the Pianka overlap values.

INCIDENCE FREQUENCY OF INSECT ORDERS

I calculated the frequency of occurrence of Lepidoptera, Coleoptera and Diptera in the sample of bat individuals. The metabarcoding output was converted to matrix containing binary presence/absence for each OTU in each individual bat. Frequency of occurrence for each prey order was then calculated as the sum of presence/absence for OTUs within that order, for each individual bat. I used a poisson generalised linear mixed-effects model to compare the frequency of occurrence or Coleoptera and Diptera between species using the *lmer()* function in *lme4*. I fitted a negative-binomial generalised mixed-effects model using the *glmer()* function in *lme4* to compare frequency of occurrence of *Lepidoptera* in bats' diets. The negative binomial distribution was used instead of the Poisson due to slight overdispersion detected in the model (ratio = 1.711, $p<0.01$). Model selection was carried out using likelihood ratio tests (LRTs) according to the method described in the statistical methods section.

ETHICS AND LICENSE STATEMENT

I was provided the dataset for this study by the Biodiversity Initiative. All data was collected prior to my analysis. I have the full permission of the authors responsible for data collection, and full credit is given. Data collection protocols used by Biodiversity Initiative to sample bats prioritised bat

welfare. All animals were handled with great care, with correct and safe handling procedures reviewed throughout fieldwork. The study sites were located within family-owned cacao farms, and Biodiversity Initiative obtained full permission from the relevant regulatory bodies and farm owners to conduct their fieldwork. Captured and handled bats followed guidelines approved by the American Society of Mammalogists (Sikes *et al*., 2011). No licence was required for this study.

RESULTS

TEMPORAL NICHE

In total, I analysed data from 586 individual bat captures (*H. Caffer*, n=5; *H. fuliginosus*, n=89; Cryptic, n=43; *H. ruber*, n=210; *R. alcyone*, n=219). The study species were most active during the early part of the night, with captures peaking between 18:00-20:00 (Figure 4). Species identity was a poor predictor of the time of night at which bats were captured, with the best-fit model accounting for only 2% of the variation in the data (R2=0.02). Only Cryptic bats differed significantly from other study species in its temporal activity, being active on average 50 minutes (±7.8 minutes) later than *H. fuliginosus* (P=0.009), 76 minutes (±7.6 minutes) later than *R. alcyone* (P=0.0267), and 65 minutes (±16.8 minutes) later than *H. ruber* (P=0.0697).

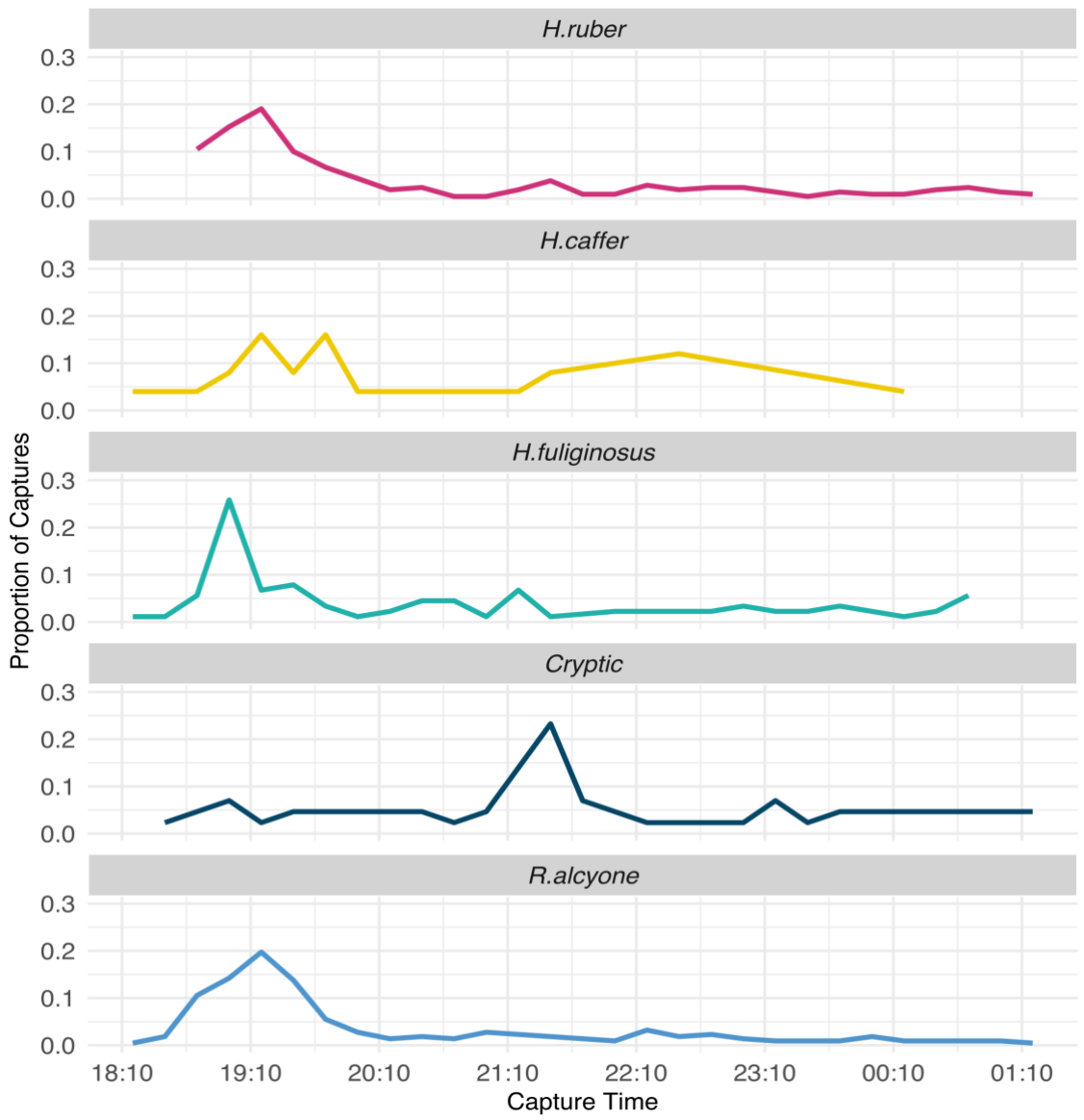

Figure 1. Time series' showing capture density for each species throughout the night. Proportion of captures measures the proportion of samples of a given species caught within a 15-minute window. Bat captures were generally highest in the early evening for most study species, except for the Cryptic bat, whose captures peaked later in the evening.

DIETARY STUDIES

The metagenomic DNA from bat faecal samples produced a total of 592,941 reads across samples from 76 *Hipposideros* individuals. The mean number of reads per individual bat was 7802 (min 2, max 79879). Mean number of reads per out/sample was 964 (min 1, max 45602). A total of 136 distinct OTUs were identified, representing 63 genera and 6 orders: *Lepidoptera*, *Diptera*, *Coleoptera*, *Neuroptera*, *Hemiptera* and *Trichoptera*. On average, 8.09 (± 0.49) OTUs were present in each sample. 73 of these OTUs occurred in just one species' diet; 25 OTUs were present in two species; 25 in three; and 13 were present in all study species' diets.

DIET COMPOSITION

Lepidoptera were the largest diet component identified (68.22% ± 3.67%) across all study species. *Diptera* (20.15% ± 2.82% of diets) and *Coleoptera* (9.25% ± 2.7% of diets) followed as other important dietary components. *Trichoptera*, *Hemiptera* and *Neuroptera* cumulatively contributed only 2.38% (± 1.06%) of diet items identified (Figure 3).

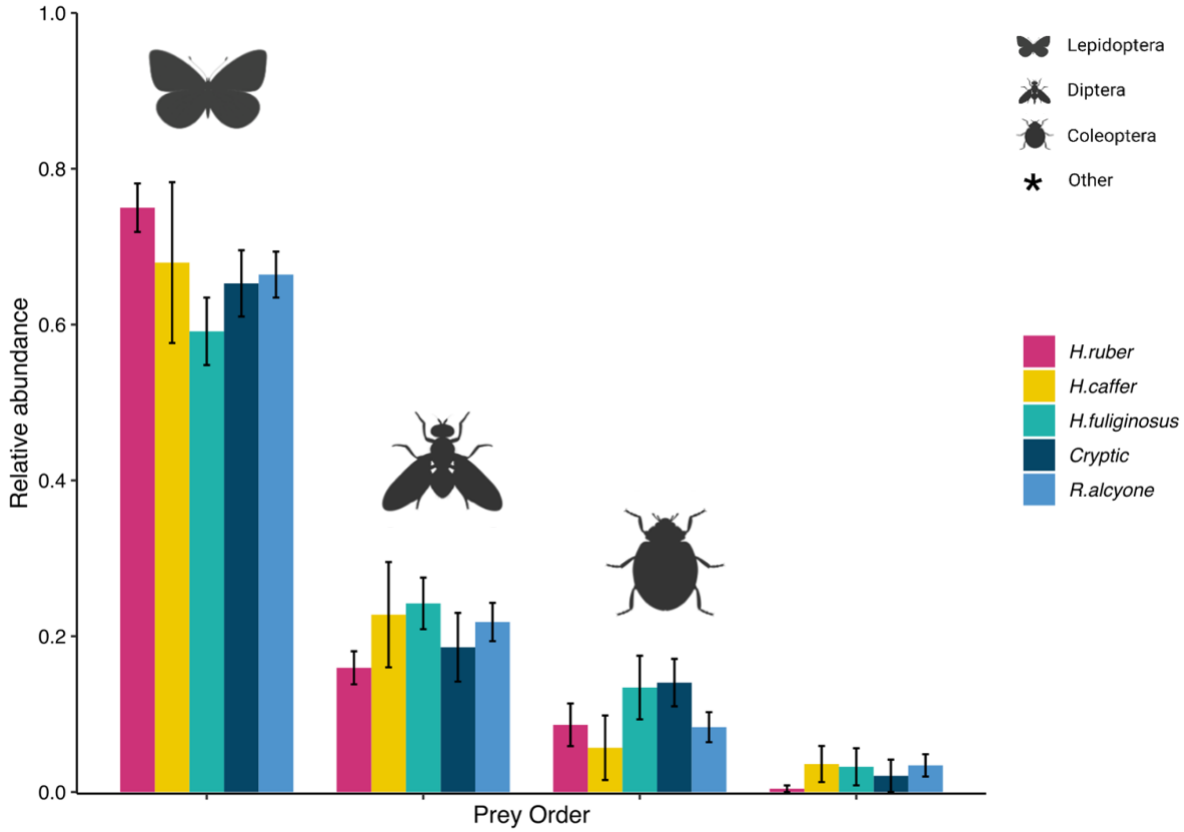

Figure 2.: Relative abundance, as proportion of total diet identified, for the four study bat species. Error bars show standard error.

DIVERSITY AND RICHNESS

Shannon's diversity index for OTU reads was 0.76 (±0.28) greater in the Cryptic bat than *R. alcyone* (p=0.0618). Reads diversity did not differ significantly for any other pairwise comparisons of species. The model explained 6% of the variation in dietary diversity in total (R^2 fixed = 0.06).

The Cryptic bat had a diet richer than *H. caffer* by 4.58 OTUs (±1.24, t=-3.698, p=0.0604), and 5.939 (±1.19) OTUs richer than *R. alcyone* (t=4.981, p=0.001). The model explained 13% of the variation in dietary richness in total ($R^{2\ fixed}$ = 0.13).

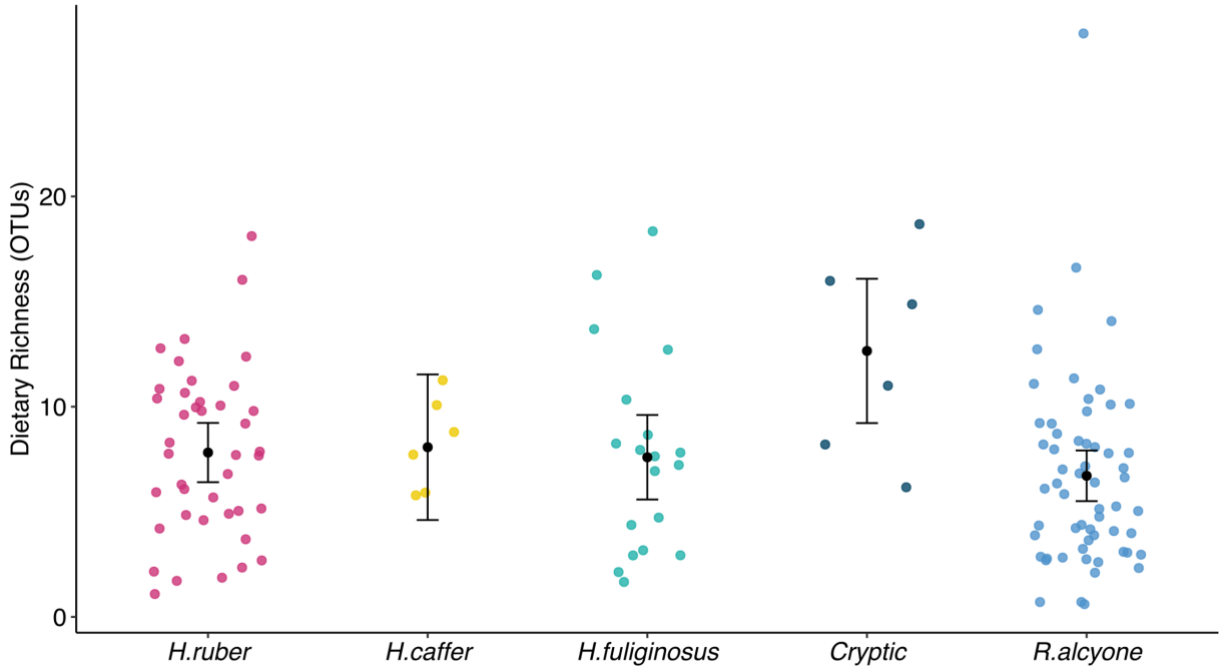

Figure 3: Dietary richness, measured as total number of OTUs detected in the faeces of an individual bat, between species. Error bars show the 95% confidence intervals as predicted by the linear model fit to the data.

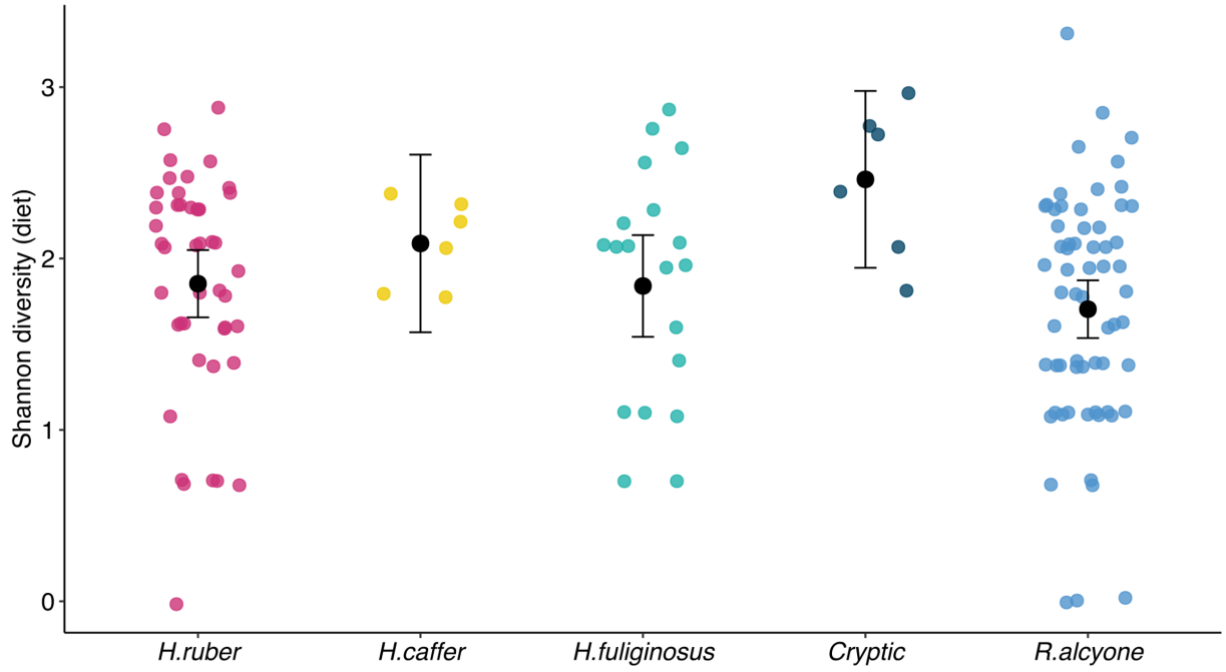

Figure 4: Dietary Diversity, calculated as Shannon's diversity index for the number of OTUs and the number of reads associated with each detected in the faeces of an individual bat. Error bars show the 95% confidence intervals as predicted by the linear model fit to the data.

DIETARY OVERLAP

The overlap of diets between all study species was significantly greater than zero (non-overlap of confidence intervals with zero). Figure 5 shows the pairwise niche overlap and confidence intervals generated over 500 bootstraps using Pianka's index. Figure 6 shows the NMDS ordination of the study species diets, and the lack of clear differentiation between any of them.

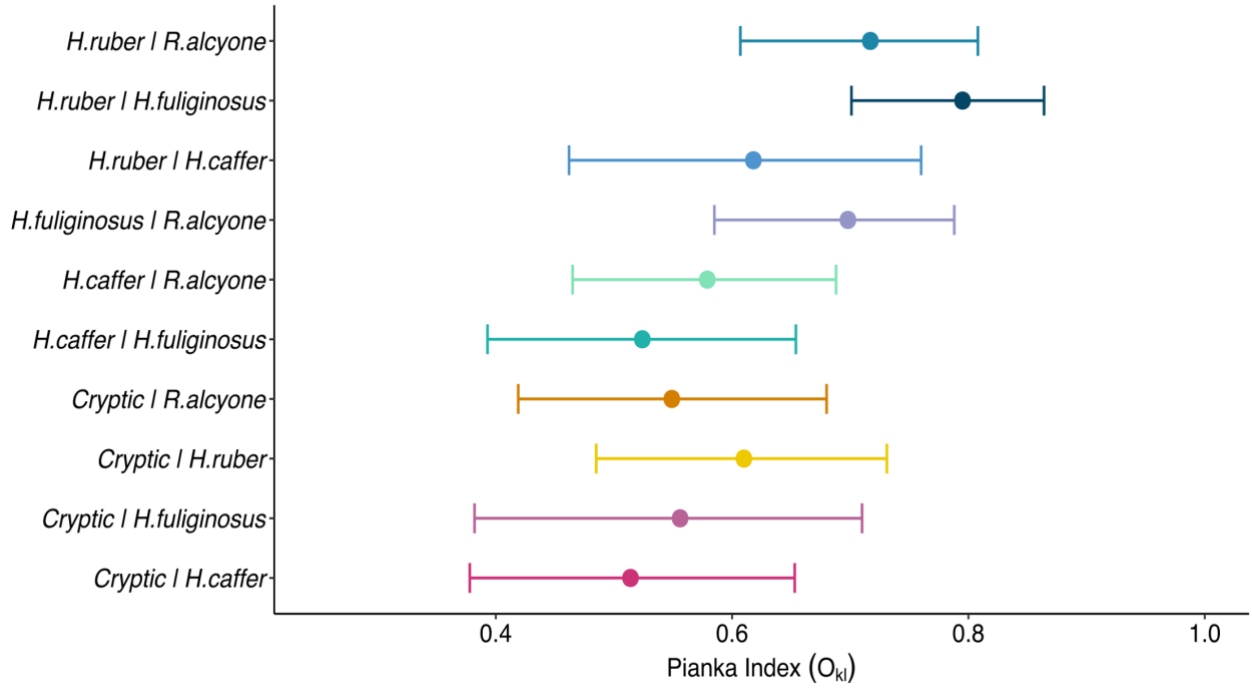

Figure 5. Pairwise dietary overlap between study species, calculated using pianka's index. Points represent the observed Pianka's index, and error bars represent 95% confidence intervals generated over 500 bootstraps.

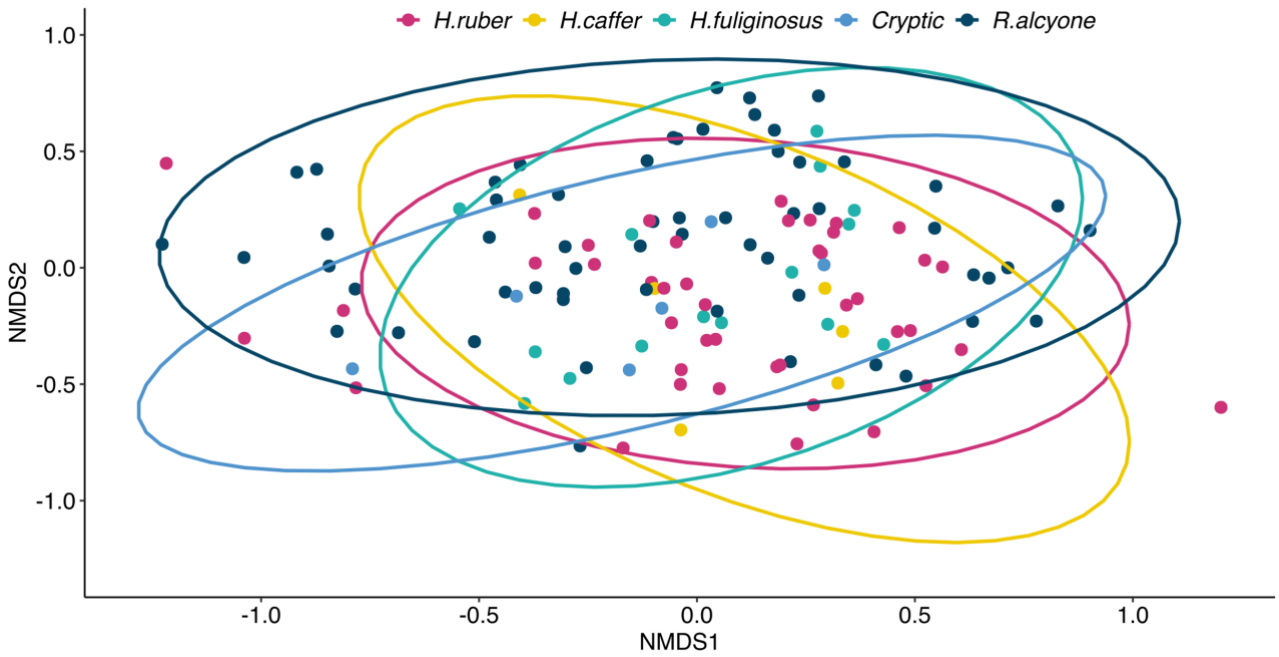

Figure 6. NMDS ordination showing broad overlap in of diet composition for all of the five study species. Each individual point represented an individual bat, with its position determined by the first two ordination co-ordinates estimated by my NMDS ordination. The ellipses represent 95% confidence intervals.

The dietary overlap between *H. ruber* and *H. fuliginosus* was significantly greater than was observed between the Cryptic bat and *H. fuliginosus*, between the Cryptic bat and *H. caffer*, between *H. caffer* and *H. fuliginosus*; between the Cryptic bat and *R. alycone*; and between *H. caffer* and *R. alcyone*. All species broadly overlapped.

Db-RDA analysis found the diet of *R. alcyone* differed significantly from *H. ruber* (SumSq=1.36, F=2.74, P=0.001), from *H. fuliginosus* (SumSq=0.815, F=1.63, P=0.01), and from the Cryptic bat (SumSq=0.774, F=1.56, P=0.008). The Cryptic bats' diets were marginally different from that of *H. ruber* (SumSq=0.652, F=1.38, P=0.058) and from *H. fuliginosus* (SumSq=0.577, F=1.371, P=0.062).

perMANOVA analysis similarly found *R. alcyone*'s diet differed significantly from *H. ruber* (SumSq=1.57, R2=0.0359, F=3.95, P=0.001), *H. fuliginosus* (SumSq=0.859, R2=0.0262, P=0.004), and the Cryptic bats (SumSq=0.791, R2=0.0285, F=1.97, P=0.011). We again found marginally significant differences between the diet of the Cryptic bat and *H. ruber* (SumSq=0.6267, R2=0.0328, F=1.662, P=0.058) and *H. fuliginosus* (SumSq=0.584, R2=0.0661, F=1.56, P=0.09).

INCIDENCE FREQUENCY OF PREY

LEPIDOPTERA

The model explained 12% of the variation in the data (R^2 =0.12), though Lepidoptera frequency of occurrence did not significantly differ between bat species. A weak signal was detected, finding the Cryptic bat to consume slightly more Lepidoptera than *R. alcyone* (Z=2.524, p=0.0852).

DIPTERA

Frequency of occurrence of Diptera did not significantly differ between the diets of the study species. The random effect included in this poisson generalised linear mixed-effects model, farm, accounted for 17% of the variation in the data.

COLEOPTERA

H. fuliginosus consumed more Coleoptera than *H. ruber* (z=-4.150, P=0.0003) and *R. alcyone* (z=2.850, p=0.0032). The Cryptic bat consumed more Coleoptera than *H. ruber* (z=-3.233, p=0.0108) and *R. alcyone* (z=2.850, p=0.0354) (Figure 8). The fixed effects in the model accounted for 17% of the variation in the data (R2=0.17).

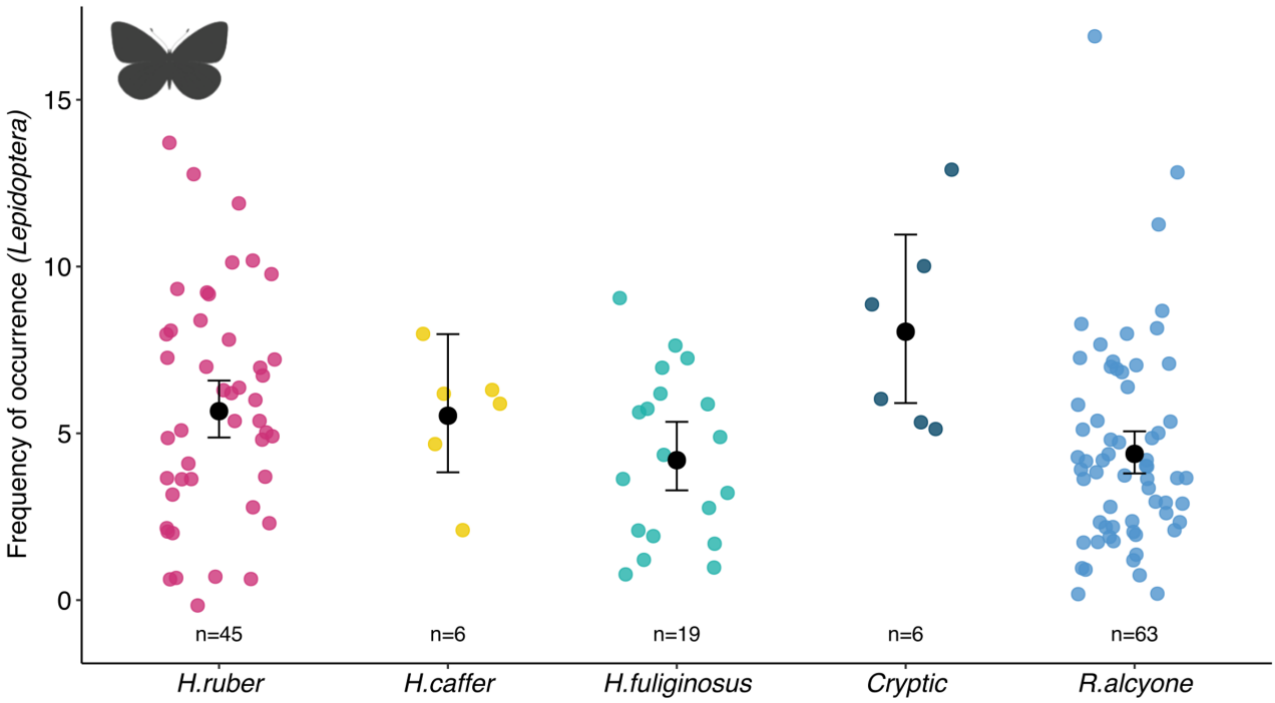

Figure 7. *Predicted occurrence (count) of Lepidoptera OTUs in bat's diets, inferred from the fitted negative binomial mixed effects models with farm as a random effect. Error bars are the 95% CI. Jittered points show raw data*

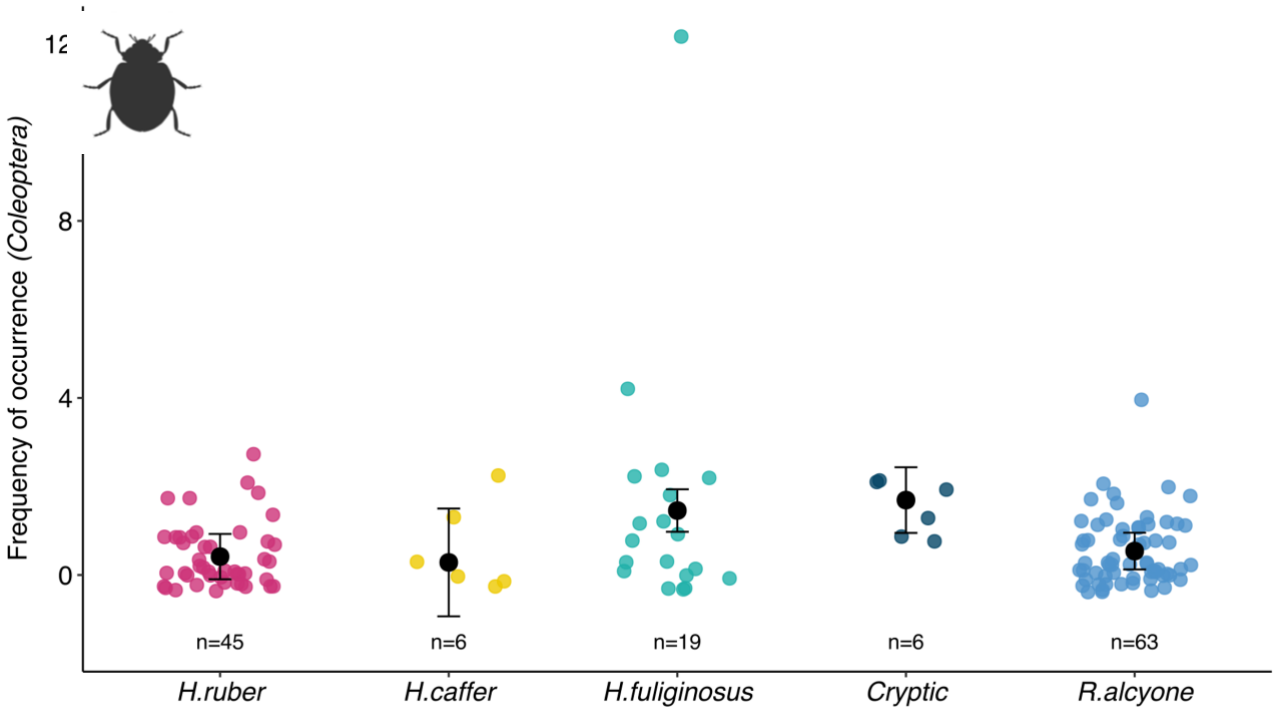

Figure 8.*Predicted occurrence (count) of Coleoptera OTUs in bat's diets, inferred from the poisson linear mixed effects model fitted with farm as a random effect. Error bars are the 95% CI. Jittered points show raw data*

DISCUSSION

TEMPORAL NICHE

With the exception of the Cryptic species, all the study species were most active during the same early period of the night between 18:00-20:00 and did not differ significantly from one another. It is unlikely that the temporal niche plays an important role in facilitating coexistence between these species. A range of ecological factors could drive the observed clustering of activity early in the evening, such as the variation in light conditions and temperature through the night. Arthropods are known to vary in their activity throughout the evening (Mata et al., 2020) and as such bat activity may simply correlate with prey abundance. Future studies seeking to test this hypothesis should perform arthropod sampling throughout the evening concurrently with bat sampling in order to test for relationships.

Interestingly, the Cryptic bat was active significantly later than three other sample species, which could be evidence of temporal partitioning from the other sample species. The dietary analyses I conducted indicated that the Cryptic bat's diet was significantly different from that of *R. alcyone*, and marginally different from *H. ruber* and *H. fuliginosus*. One plausible explanation for their later activity time could be due to increased abundance of their preferred prey items during the latter part of the night, which might be tested through sampling arthropod abundance through the night. Alternatively, Cryptic bats might have been active later to reduce competitive interactions with other study species, which could be explored by testing whether this later temporal pattern is persistent in populations of the Cryptic bat that are isolated from the other species. However, as the Cryptic bat remains poorly defined and has only been identified in landscapes where it co-occurs with the other species, this study is not yet possible.

DIETARY NICHE BREADTH

Segregation along a specialist-generalist axis is another potential stabilising mechanism contributing to bat coexistence. I used both dietary species richness and Shannon's diversity index to estimate dietary breadth. Dietary species richness is a count of the total distinct OTUs present in an individual's diet, whilst Shannon's diversity index also considers the abundance of each prey item in diet, estimated as the number of reads.

The Cryptic bat's diet was significantly richer than that of *H. caffer* and *R. alcyone*, whilst other pairwise comparisons in dietary richness did not find significant differences. The Cryptic bat's diet was also more diverse than that of *R. alcyone* but was not significantly more diverse than any other species. Other pairwise comparisons were not significantly different. This tentative evidence of a more generalist diet in Cryptic bats than other species could be more robustly tested through

comparison with insect abundances through the night to determine whether they are feeding less selectively than other species.

NICHE OVERLAP (PERMANOVA, NMDS, PIANKA, DB-RDA)

NMDS ordination showed broad overlap between all the study species' diets. This was further supported by Pianka's index calculations, with all pairwise comparisons between species overlapping significantly. This is unsurprising for members of the same trophic guild feeding in the same landscape. Finer-scale tests of overlap were carried out using Db-RDA analysis and perMANOVA. Both methods found *R. alcyone*'s diet to be significantly different from *H. ruber*, *H. fuliginosus*, and Cryptic bats. The Cryptic bat's diet was marginally different from that of *H. ruber* and *H. fuliginosus* according to both methods.

R. alcyone was the lone representative of the family *Rhinolophidae* in the study. The pattern of *R. alcyone* differing from the *Hipposideros* bats suggests that dietary niche segregation may be more pronounced between taxonomic groups than it is within genera, as there were no significant differences in the diets of *Hipposideros* bats in the study. To assess this further, future studies should include a broader range of species from both the *Hipposideridae* and *Rhinolophidae* and compare the effect sizes of dietary overlap within and between these taxa.

The marginal difference of the Cryptic bats diet again provides further evidence for a distinct trophic niche for these bats.

FREQUENCY OF OCCURRENCE

The frequency of occurrence of the two most important prey taxa, *Lepidoptera* and *Diptera*, did not vary significantly between any of the study species. This is surprising given the apparent dietary partitioning of some species revealed in the Db-RDa and perMANOVA analyses on diet overlap. This suggests that the differences driving this diet partitioning must occur at a finer scale than at order level. Future study could address this gap using a larger sample size allowing for more robust identification of OTUs to greater taxonomic resolution.

Species showed differences in their predation upon *Coleoptera*, with both *H. fuliginosus* and the Cryptic bats consuming more *Coleoptera* than *H. ruber* and *R. alcyone*. As the primer used in metabarcoding, ZBJ, is not designed for effectiveness in sequencing coleoptera (Zeale et al., 2011), which highlights a potential limitation of my study.

LIMITATIONS

The dietary analyses conducted in my study relied solely upon the outputs of metabarcoding runs, and hence was strongly impacted by the limitations of this method.

A substantial limitation of using metabarcoding data for dietary analysis in insectivores is that I cannot differentiate between life history stages of prey. Insects vary substantially throughout their life cycle in size, habitat and behaviour. Specialisation toward a particular life history stage, such as gleaning for larvae from vegetation versus actively hunting adult insects in flight, cannot be detected in my analysis and therefore I missed a potential axis for dietary segregation. Future studies on under-studied bats such as my study species, for which the primary foraging strategy is not known will encounter the same challenge and should consider the trade-off between the scale and resolution offered by DNA metabarcoding versus the many challenges of observational studies on bat feeding.

The Db-RDA analysis I conducted comparing dietary overlap was run using presence/absence matrices for prey items, giving equal weighting to prey items regardless of how abundant they were in diets. This may have masked the relative importance of prey items when calculating the degree of overlap. This was mitigated somewhat by the complementary perMANOVA analysis I ran for the same pairwise comparisons, which found broadly similar contrasts between groups.

Relying upon OTUs to infer prey is likely to have inflated the importance of species rich taxonomic groups compared to less species rich prey taxa. Insect richness is incredibly high in tropical forests, and presence of two sister prey species in a bats diet is less meaningful in terms of niche segregation than the presence of two functionally different prey species that might represent totally different classes of prey item. However, my dietary overlap analyses were conducted at the OTU level and so were not able to make such distinctions. Future studies might account for this focussing on the functional traits of prey to inform overlap analyses. I was only able to identify OTUs to order level reliably which offers little functional information; future studies would require a metabarcoding approach capable of identifying OTUs more reliably to genus or family level in order to infer more about their functional traits.

The ZBJ primer was selected for its ability to amplify *Lepidoptera* and *Diptera* sequences (Zeale et al., 2011), which were known to be major diet elements in the study species. However, this restricted my ability to test for fine-scale differences in the occurrence of other insect orders in bat diets, and to detect potential segregation of diet across these other orders between bat species.

Prey availability is known to impact upon the degree of dietary segregation between species (Aizpurua et al., 2018b) Unfortunately, due to limitations in the arthropod surveys conducted during the bat sampling, I was unable to compare prey abundance with occurrence in the bats' diets. Future

studies on these sites could investigate the impact of prey abundance upon prey selectivity by conducting regular arthropod surveys throughout the night.

The Metabarcoding runs were conducted prior to this study's design. As a result, the sample sizes available were not optimal for comparison between the groups in this study. Both the Cryptic bat and *H. caffer* were represented by only 6 individuals. A larger sample size would not only improve the reliability of the data but allow deeper investigation into the impact of landscape and farm, for which the sample sizes would have been too small to run meaningful analyses.

In order to be more confident in the dietary segregation I found between multiple species, future studies might consider comparing the diets of these species in allopatry versus sympatry, in a similar manor to the methods described by Arrizabalaga-Escudero et al., (2018) in their study of European bats. If the observed dietary differences are driven by interspecific competition, then I would expect the dietary segregation to be reduced when the bats are not co-occurring.

CONCLUSION

This study aimed to narrow the substantial knowledge gap around the mechanisms that facilitate bat species coexistence at the high levels found in the tropics. I tested for evidence of niche partitioning in a group of sympatric insectivorous bats sampled on Cacao farms in Cameroon, from the genera *Hipposideros* and *Rhinolophus*. The study set out to address the following research objectives:

(1) Do species display different temporal activity patterns?

(2) Do species differ in their dietary breadth?

(3) Do species diets overlap with each other?

(4) Are there fine-scale differences in the dietary composition of different species?

Data from DNA metabarcoding of bat faeces was used to provide insight into the dietary niche objectives.

I found that whilst most species do not differ in their temporal activity, a newly identified Cryptic population was most active significantly later than closely related members of the same community. This same cryptic population appears to have a broader, distinct diet compared to its close relatives, consisting of more *Coleoptera*. However, small sample size and limited metabarcoding resolution limit any inferences into the fine-scale differences that drive this segregation.

Additionally, a pattern emerged with the diets of bats within the genus *Hipposideros* typically not differing from each other but differing significantly from a representative of a sister taxon, *Rhinolophus alcyone.* This suggests that dietary niche segregation is more pronounced between

genera than within them in this community, and supports the assumptions made in previous studies that phylogenetic relatedness predicts reduced intraspecific competition owing to a recent shared niche history.

The results of this study contribute to our growing understanding of bat coexistence. Perhaps most interestingly, it highlights that temporal activity through the night can vary between closely related species. This has implications for future coexistence studies in bats, which seldom consider temporal partitioning as a potential axis for specialisation, or a driver in the emergence of cryptic populations.

Future study should look to study these species in allopathy as well as in sympatry, to determine whether the observed differences are the result of interspecific competition. Furthermore, future research should gather arthropod abundance data concurrently with bat samples and at different times throughout the night, to determine the extent to which bats select their diets as opposed to simply predating upon the most abundant prey at a given time.

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