THESIS SUMMARY

Sound Based Predator-Prey Interactions Between European Bats and Bush-Crickets

By

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Dedication

This work is dedicated to my mother, Elizabet Valkova-Hubancheva, for her love of science, knowledge and the beauty of life.

Посвещение

Посвещавам този труд на майка ми Елизабет Вълкова-Хубанчева.
Заради любовта към знанието, науката и красотата на живота.

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Резюме

Разнообразието и сложността на наземните екосистеми до голяма степен зависят от взаимодействията между хищниците и техните жертви. Тези взаимоотношения влияят върху разпространението, изобилието и разнообразието на видовете в екосистемите и поради това са обект на изследвания от векове. Но за да разберем напълно как точно протичат тези взаимоотношения и какви са последиците от тях, е важно да знаем контекста на сетивния свят, в който те се развиват. Така, например, хищниците притежават специализирани сензорни системи, които им помагат в преследването и улавянето на плячката, докато сетивата на потенциалните жертви са пригодени за засичане и избягване на хищнически атаки. Акустичните сигнали играят важна роля в тези взаимодействия, влияейки на поведението и еволюцията на множество организми.

Тази докторска дисертация се фокусира върху звуковите взаимодействия между европейските прилепи голям и остроух нощник (*Myotis myotis* и *Myotis blythii*) и техните жертви – дългопипалните скакалци от семейство *Tettigonidae*. Изследванията включват изясняване на хранителната база на двата вида прилепи чрез ДНК метабаркодиране, проследяване на движенията и хранителното поведение на големите нощници с помощта на миниатюризирани акустични сензори и изследване на стратегиите за избягване на хищници на дългопипалните скакалци, докато привличат брачни партньори.

Взети заедно, тези изследвания хвърлят светлина върху механизмите, които обуславят взаимодействията между хищниците и тяхната плячка и еволюционните сили, които им влияят. Резултатите от тази дисертация подчертават потенциала на комбинирането на различни интердисциплинарни подходи, като молекулярни техники, проследяване и поведенчески експерименти, за да се подобри разбирането ни за заобикалящия ни свят. В следващите глави са изложени основните открития, резултати и заключения от изследванията, включени в дисертацията, и е обсъдено как те допринасят за по-доброто разбиране на поведението и еволюцията на взаимодействията между хищници и плячка в естествените биоми.

I. Introduction

Predator-prey interactions play a crucial role in shaping the diversity and abundance of species in terrestrial ecosystems (Abrams, 2000). These interactions involve a range of sensory-based adaptations that allow predators to pursue and capture their prey, while prey species have developed sophisticated mechanisms to evade capture (Stevens, 2013). Examples include prey species with olfactory or taste-based defences, rendering them unpalatable or toxic to predators, and those using bright coloration or sound signals to warn potential predators (Caro, 2005; Leavell et al., 2018; Krivoruchko et al., 2021). Predators, on the other hand, rely on diverse sensory mechanisms to detect, track, and capture their prey (Stevens, 2013).

In this intricate web of sensory-based interactions, my thesis hones in on a specific yet critical channel – the acoustic sense. Sound perception and emission in predator-prey interactions are instrumental, shaping the behaviour and evolution of both predators and prey (Fletcher, 2014). Understanding sound perception is crucial for comprehending various aspects of animal life, including foraging, navigation, mate selection, territorial defence, and predator avoidance (Fenton et al., 2016). The thesis focuses specifically on sound-based predator-prey interactions between European bats and bush-crickets, exploring the diet of bats through DNA metabarcoding, tracking the movements and foraging behaviour of bats using miniature tags, and investigating the antipredator strategies of bush-crickets through behavioural experiments.

The findings of the thesis shed light on the intricate mechanisms and evolutionary forces driving predator-prey interactions in complex ecosystems. They also underscore the potential of combining different disciplinary approaches, like molecular techniques, biologging, and behavioural experiments, to enhance our comprehension of the natural world. The following chapters will further delve into these explorations, their key findings, and their implications, all contributing to a better understanding of the ecology and evolution of predator-prey interactions in natural communities.

II. Aim and objectives

The aim of this thesis is to elucidate the sensory and behavioural mechanisms of prey detection by predators and of defence strategies by prey in the complex predator-prey system between the lesser and greater mouse-eared bats (*M. myotis* and *M. blythii*) and the bush-crickets of the family Tettigoniidae.

Main objectives

1. Determine the diet of *M. myotis* and *M. blythii* by identifying the composition of their faeces using an integrated approach.
2. Investigate the acoustic cues in the mating calls that are exploited by bats for locating singing bush-crickets.
3. Explore the sensory and behavioural defence mechanisms of bush-crickets against eavesdropping bats.

III. Main chapters

Chapter 1. DNA metabarcoding data from faecal samples of the lesser (*Myotis blythii*) and the greater (*Myotis myotis*) mouse-eared bats from Bulgaria

Abstract

A comprehensive understanding of trophic interactions in terrestrial ecosystems is crucial for ecological research and conservation. Recent advances in non-invasive methods, such as environmental DNA (eDNA) metabarcoding, have enabled researchers to collect vast amounts of data on wild animal diets. However, sharing this data and metadata effectively and transparently presents new challenges. To address this, a new type of scholarly journal publication has emerged that aims to describe datasets rather than report research investigations. In this paper, we present a dataset of consumed prey species and parasites based on the metabarcoding of 113 faecal samples from the greater and lesser mouse-eared bats (*Myotis myotis* and *Myotis blythii*), along with a detailed description of the data sampling, laboratory analysis, and bioinformatics pipeline. Our dataset comprises 1018 unique Barcode Index Numbers (BINs) from 12 Classes and 43 Orders. This data can be used for meta-analysis, exploring new predator-prey and host-parasite interactions, studying inter- and intraspecific ecological interactions, and informing protected area management, among other applications. By sharing this dataset, we hope to encourage other researchers to use it to answer additional ecological questions and advance our understanding of trophic interactions in terrestrial ecosystems.

Methods

To create the dataset we used DNA extraction, high-throughput sequencing, pre-processing of sequencing data, bioinformatic analysis, and taxonomic annotation of faecal samples collected from wild mouse-eared bats (*60 M. myotis* and 53 *M. blythii*) from Bulgaria.

Results and Discussion

The results indicate that insects comprise the largest proportion of the detected species in the dataset. The relative read abundance (RRA) of carabid beetles differed significantly between *Myotis myotis* and *Myotis blythii*, reflecting their differences in diet, foraging style, and habitat preferences. The Krona charts also revealed differences in the RRA of Orthopteran species between the two bat species, with crickets being more prevalent in *M. myotis* samples and bush-crickets dominating in *M. blythii* samples. These findings align with the known foraging strategies of the two species. It is important to note that the RRA does not directly represent biomass abundance and should be only interpreted as a guide to taxonomic diversity and relative abundance of the reads not as a quantitative information.

In addition to prey species, the dataset includes reads from various ecto- and endo-parasites, as well as molluscs and worms (consumed by the carabid beetles). These findings suggest that metabarcoding can be used for the study of complex trophic interactions.

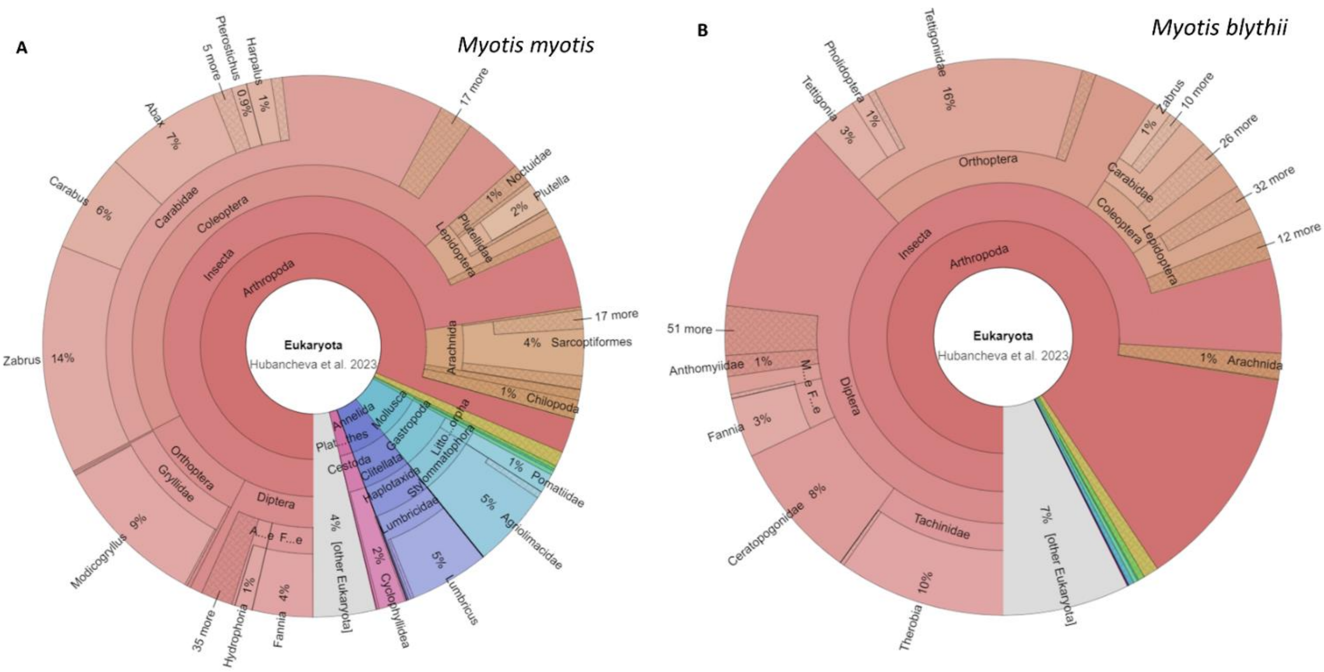

Fig. 1 Taxonomic relationships and relative abundance of prey and parasite species in faecal samples collected from **A)** 60 individuals of the greater mouse-eared bat, *Myotis myotis*, and **B)** 53 individuals of the lesser mouse-eared bat, *Myotis blythii*.

Chapter 2. Echolocating bats prefer a high risk-high gain foraging strategy to increase prey profitability

Abstract

Predators that target multiple prey types are predicted to switch foraging modes according to prey profitability to increase energy returns in dynamic environments. Here, we use bat-borne tags and DNA metabarcoding of feces to test the hypothesis that greater mouse-eared bats make immediate foraging decisions based on prey profitability and changes in the environment. We show that these bats use two foraging strategies with similar average nightly captures of 25 small, aerial insects and 29 large, ground-dwelling insects per bat, but with much higher capture success in the air (76%) vs ground (30%). However, owing to the 3–20 times larger ground prey, 85% of the nightly food acquisition comes from ground prey despite the 2.5 times higher failure rates. We find that most bats use the same foraging strategy on a given night suggesting that bats adapt their hunting behaviour to weather and ground conditions. We conclude that these bats use high risk-high gain gleaning of ground prey as a primary foraging tactic, but switch to aerial hunting when environmental changes reduce the profitability of ground prey, showing that prey switching matched to environmental dynamics plays a key role in covering the energy intake even in specialized predators.

Methodology

The study combines several methods including biologging, captive experiments, and DNA metabarcoding.

Results

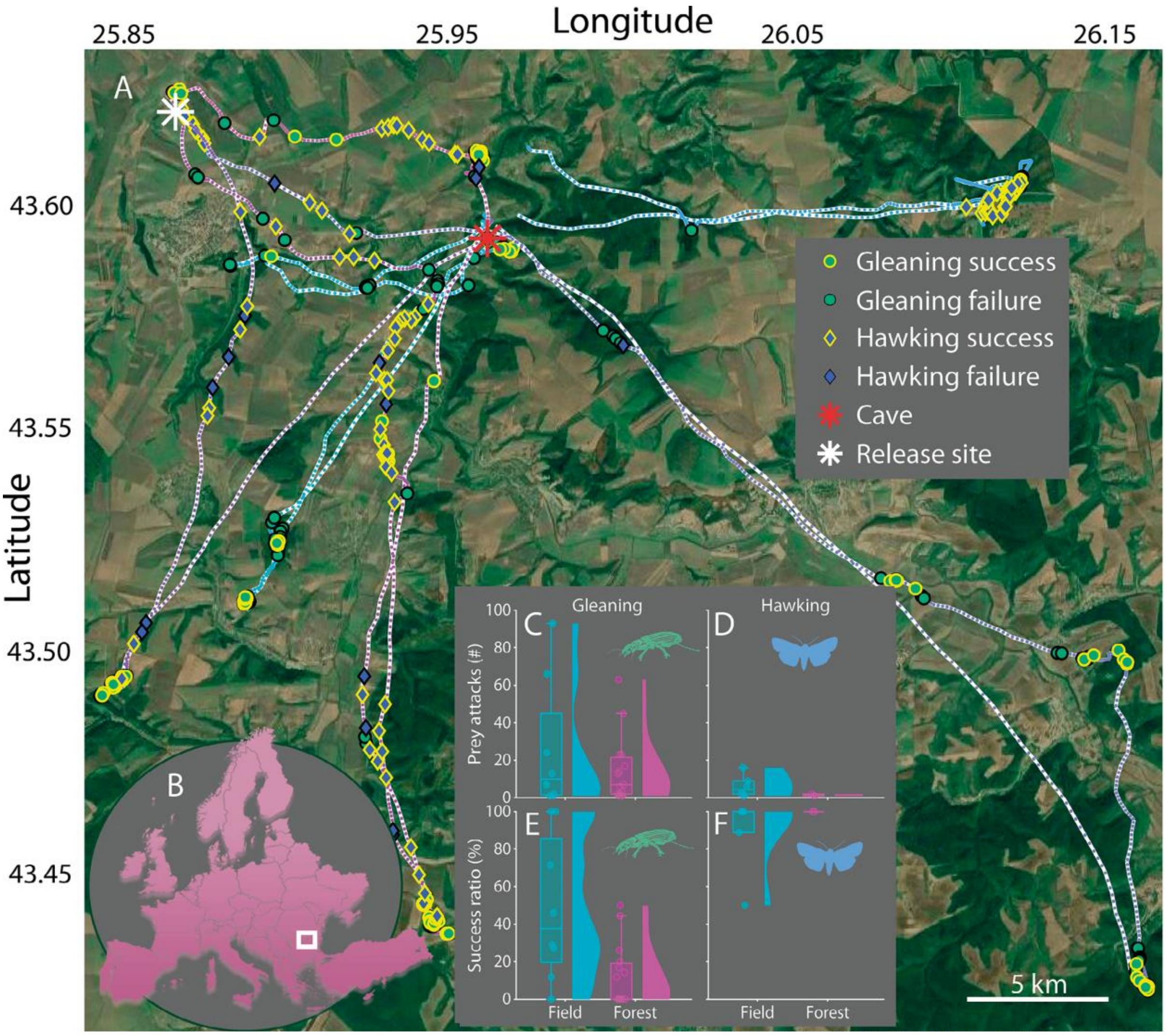

Figure 1. Habitat influences the foraging success of greater mouse-eared only when gleaning. (A) Tracks of seven bats with GPS tags released either at the cave (red star) or at a location nearby (white star) and their foraging behavior: Gleaning (green circles) and hawking (blue diamonds) attacks along with their success (yellow edge) or failure (black edge). (B) The bats were tracked in North-Eastern Bulgaria (white square). C-F: Total prey attacks (CD) and success ratios per foraging bout (EF), for both habitats: open field (blue; G) and forest (magenta; H). Each data point corresponds to one foraging bout.

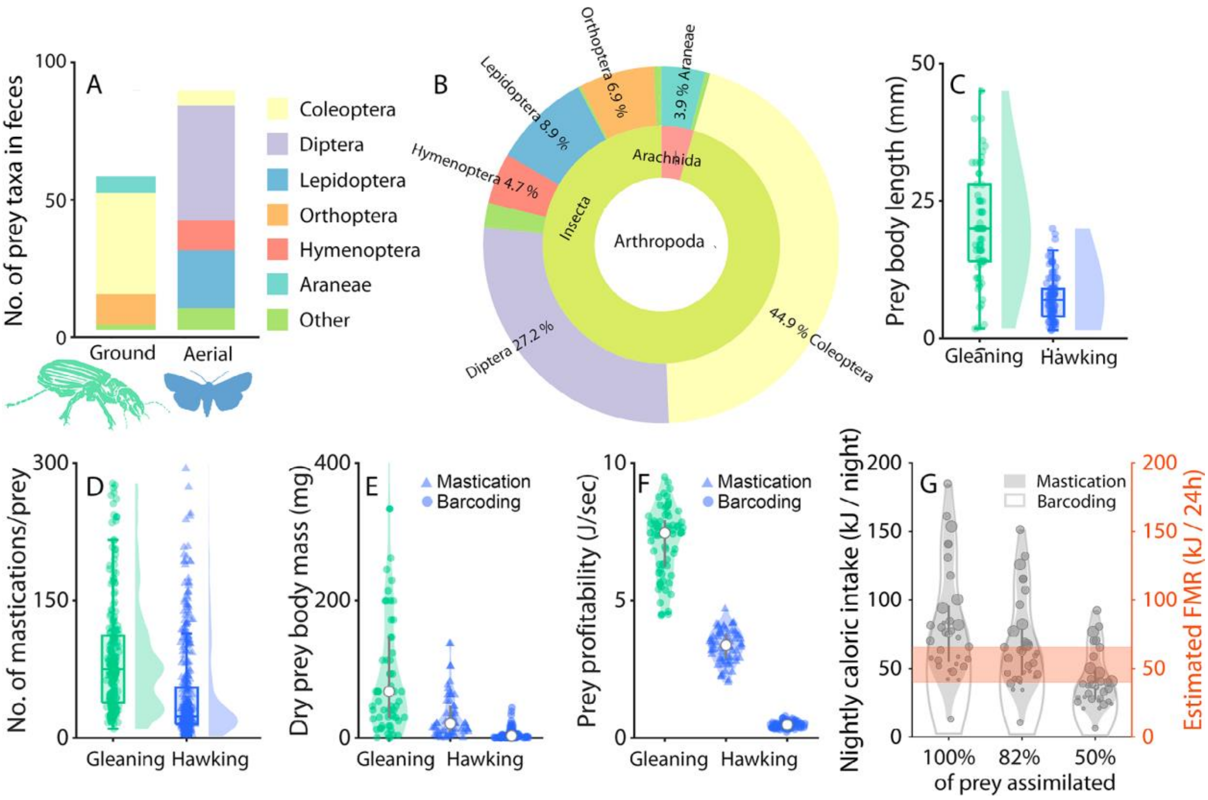

Figure 3. Ground prey is larger than aerial prey and sufficient to offset the lower foraging success ratios of gleaning. (A-B) DNA metabarcoding of feces from 54 greater mouse-eared bats (48 females, six males). Insects were categorized as either ground (green) or aerial (blue). The few prey species (N=5) that are both aerial and ground were omitted from the analysis. Distribution of the targeted prey orders depicted as OTU (Operational Taxonomic Units) between ground (~40%) and aerial (~60%) niches (A) and across taxonomical units in the Arthropoda (B). (C-F): Prey properties and profitability during gleaning (ground prey, green) and aerial hawking (aerial prey, blue), with kernel densities and boxplots. (C) Body lengths of the prey sorted by foraging strategy. (D) Number of mastication sounds identified after each prey capture by an automatic detector (N=244 ground captures and 336 aerial captures across 10 bats). (E) Dry prey body masses of each prey type identified for gleaning via DNA metabarcoding (green circles) (DNA metabarcoding was used as the reference prey body mass for ground captures), and for aerial prey by mastication analysis (blue triangles) and DNA metabarcoding (blue circles). (F) Prey profitability of gleaning or hawking prey calculated from prey body masses from mastication analysis (triangles) or DNA metabarcoding (circles) combined with observed success ratios, handling, and search times. The data are plotted for bootstrapped data (N=70 random data points) due to varying sample sizes of each parameter. (G) Total caloric intake per night per bat calculated by multiplying the caloric intake per prey with the number of successful gleaning and hawking prey captures, and compared to the field metabolic rate of a 30 g bat estimated from the literature (orange).

Chapter 3. Bush-crickets show lifelong flexibility in courtship signals to match predation threat

Abstract

The conflicting selection pressures of advertising to conspecifics and inadvertently advertising to predators has direct fitness consequences. It is unclear, however, how signalling prey species balance costs and benefits of those vital tasks over their lifetime. To address this, we quantified how a prey species with a conspicuous courtship song, the bush-cricket *Tettigonia viridissima* (Orthoptera: Tettigoniidae), adjusts its singing behaviour in response to increasing threat levels of bat predation and how those adjustments change in the course of its lifetime. We show that young males favour predator avoidance over mating by acoustically hiding from predators for a longer time, while old males prioritize mating opportunities over predator avoidance. Thus, males of different ages trade-off differently the risk of losing mating opportunities against the risk of falling prey to bats. This illustrates that prey species are capable of carefully balancing the costs and the benefits of reproduction and survival throughout their lifetime. Our results highlight the flexibility of vital behaviours and demonstrate how they are balanced over a lifetime to maximize fitness.

Methods

In this study we conducted playback experiments on male bush-crickets to investigate changes in their anti-predator behaviour based on perceived predation risk and lifespan. Different sound stimuli were presented, and the bush-crickets' singing behaviour was recorded. Statistical modelling were used to analyse the data and assess the effects of predation threat and age.

Results

In the study, male bush-crickets were tested to examine their response to predation threat and age in terms of their courtship behaviour. The results showed that as the level of predation threat increased, the likelihood of males stopping their courtship song also increased. Age did not have a significant effect on the likelihood of stopping singing. Furthermore, the duration of pauses in the courtship song increased with higher predation threat, but old males had shorter pause durations compared to young males. Additionally, most males resumed singing early in response to low predation threat or the control sound resembling an innocuous cricket song, indicating a lower perceived danger. These findings demonstrate that male bush-crickets adjust their anti-predator behaviour in response to predation threat, but age influences the overall response strength.

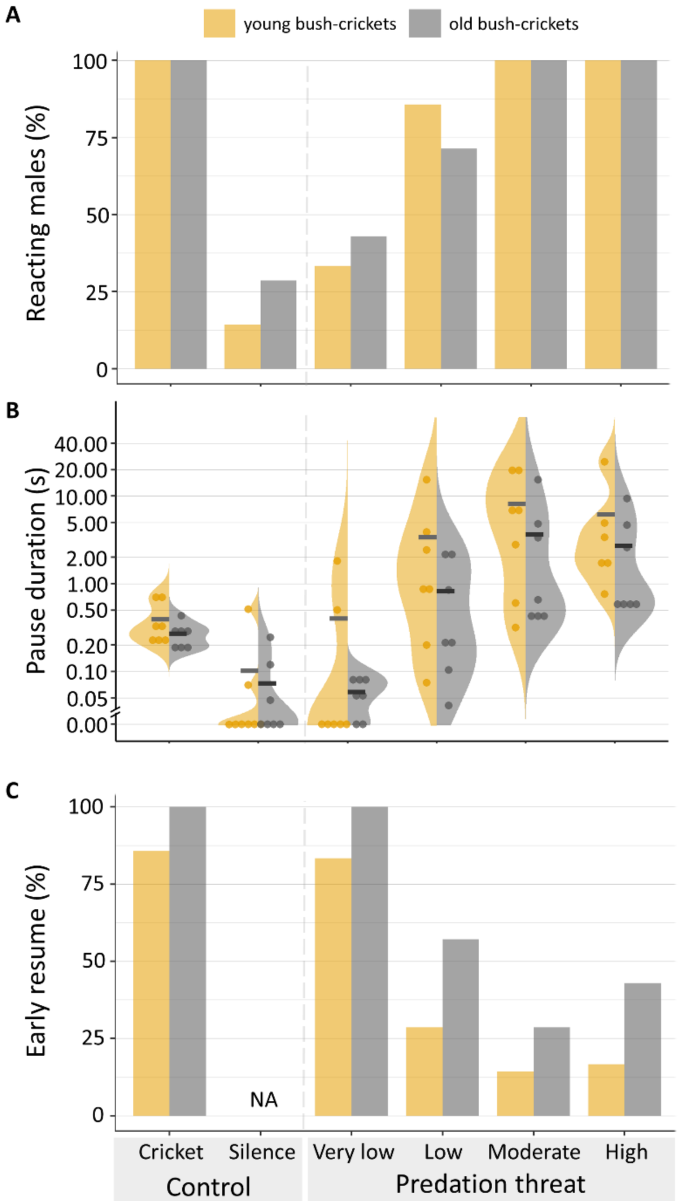

Figure 1. Singing male bush-crickets adjust their anti-predator behaviour to predation threat and age. Individual bush-crickets received six acoustic treatments of 960-ms duration each: two control sounds, one resembling the song of a sympatric innocuous cricket (88 dB SPL RMS re. 20 µPA) and the other one being silence, and four levels of predation threat (from 'very low' to 'high') consisting of sequences of bat-like calls with increasing sound pressure levels (57, 67, 77 and 87 dB SPL RMS re. 20 µPA). Data are shown separately for young (yellow, N=7) and old males (grey, N=7). A) The percentage of males that stopped singing their courtship song increased with increasing predation threat, but was independent of age. B) The pause duration of the song increased with increasing predation threat, and was shorter for old than young males. Split violin plots show raw data points, mean values (thick dark lines) and density curves (Kernel density estimation with 0.9 smoothing bandwidth). C) The percentage of singing males that resumed singing before the end of the bat attack increased with lower predation threat, and was higher for old than young males.

IV. Summary and main conclusions

Summary

This thesis aimed to uncover the intricate sensory and behavioural mechanisms underlying prey detection by predators and the defence strategies employed by prey in the predator-prey system of the lesser and greater mouse-eared bats (*M. myotis* and *M. blythii*) and their prey, bush-crickets (family *Tettigoniidae*). The primary objectives of this research were to determine the dietary preferences of *M. myotis* and *M. blythii* through an integrated approach, investigate how bats exploit the acoustic cues in bush-crickets' mating calls to locate singing males, and explore the sensory and behavioural defence mechanisms of bush-crickets against eavesdropping bats. By addressing these objectives, my thesis enhances our understanding of the sensory and behavioural dynamics involved in the predator-prey interactions between bats and bush-crickets and expands our knowledge of the ecological intricacies within this predator-prey system.

Objective 1

The first objective of this dissertation was to determine the species composition of the prey of the greater and lesser mouse-eared bats. It was performed through an integrated approach involving the collection and analysis of faecal samples, the use of miniaturised computer tags, and behavioural experiments in the laboratory.

Faecal samples were collected from 113 individual bats (60 *M. myotis* and 53 *M. blythii*) from June to August 2017 and 2018. The samples were stored in 98% ethanol until the subsequent DNA extraction, PCR amplification of the CO1 gene, high-throughput sequencing of the amplicons, and bioinformatic analysis, to identify the taxonomic identity of the amplified DNA sequences by comparing the data to the BOLD and NCBI Genbank databases and the Ribosomal Database Project Classifier algorithm. The results from the metabarcoding analysis suggest that insects made up the largest proportion of the detected prey species. The observed differences in the relative read abundance (RRA) from carabid beetles between *M. myotis* (46%) and *M. blythii* (7%) aligns with the differences in diet, foraging style and habitat of these species (Arlettaz 1996). However, the differences in the RRA of other taxa shown in the Krona charts were less pronounced between the two bat species, with an important exception being Orthoptera. In *M. myotis* samples, the majority of Orthopteran species belonged to the family Gryllidae (crickets), while in *M. blythii* samples, the records primarily came from the family

Tettigoniidae (bush-crickets), including species such as *Tettigonia*, *Phaneroptera*, *Pholidoptera*, *Decticus*, *Poecilimon*, and *Isophya*. This observation aligns with the known differences in foraging strategies between the two species, where *M. myotis* primarily utilizes rustling sounds produced by moving insects (incl. crickets), while *M. blythii* eavesdrops on the mating songs of bush-crickets (Arlettaz 1996; Jones et al., 2010; Stidhsold et al., 2023). Notably, in addition to the bats' prey species, the provided dataset also includes reads from various ecto- and endo-parasites, such as ticks (*Ixodes*), mites (Mesostigmata and Sarcoptiformes), roundworms (Strongylida and Rhabditida), and other parasite species. Furthermore, we identified molluscs (Gastropoda) and worms (Annelida) in the samples, including *Pomatias rivulare*, *Lumbricus rubellus*, and *Eisenia fetida*, which were likely consumed by predatory carabid beetles or other arthropods that were then consumed by the bats. Moreover, the presence of species from the roundworm genus *Steinernema*, which are known to parasitize mole-crickets and other bat prey, suggests that the dataset also contains parasites of the bats' prey species.

In the next step, by combining the metabarcoding techniques with behavioural experiments and biologging of wild bats, we studied the diet and the hunting behaviour of the greater mouse-eared bat (*M. myotis*) in unprecedented detail. We used miniaturised biologging devices to track the hunting behaviour of 34 bats. These tags recorded the bats' echolocation behaviour, three-dimensional movement patterns, GPS locations (N=7 of 34 bats) and mastication sounds after prey captures as a proxy for foraging success. In addition, captive bats were observed capturing prey in realistic settings but under controlled lab conditions using microphone arrays and high-speed cameras. Our results show that the greater mouse-eared bat use two foraging strategies with similar average nightly captures of 25 small, aerial insects and 29 large, ground-dwelling insects per bat, but with much higher capture success in the air (76%) versus ground (30%). However, owing to the 3–20 times larger ground prey, 85% of the nightly food intake comes from ground prey despite the 2.5 times higher failure rates.

In summary, this dissertation effectively combined metabarcoding analysis and behavioral experiments to unravel the dietary preferences and foraging strategies of greater and lesser mouse-eared bats. The DNA analysis uncovered distinct diet variations that aligned with their established behaviours, while behavioral tracking illuminated the bats' dual hunting strategies, highlighting a preference for ground prey despite higher failure rates. This combined approach offers a comprehensive understanding of these bats' feeding behaviours, revealing the intricate interplay between their diet and hunting tactics.

Objective 2

The second objective of the thesis was to investigate the acoustic cues in the mating calls of katydids that are exploited by bats for locating singing males. While previous studies have offered indirect insights through behavioural experiments in captivity and diet analyses, demonstrating that bats eavesdrop on the mating songs of bush-crickets, direct evidence of this phenomenon remains elusive. To achieve this objective, miniaturised biologging devices were used to study the acoustic perceptual field of greater mouse-eared bats. The tags recorded sound with an ultrasonic microphone and sampled the bat's behaviour by synchronised tri-axial accelerometers and magnetometers (Stidsholt et al. 2018). A total of 27 wild greater mouse-eared bats were tagged and released the following night at a field near the roost. From 3917 attacks on prey recorded in total, approximately two-thirds were attacks on ground-dwelling prey species. The microphone sensitivity of the tags and the low signal-to-noise ratio of the recordings allowed for an in-depth analysis of the prey sounds and echoes received by the bat, to understand its foraging behaviour. Our results show that almost all of the ground attacks were not initiated in response to detectable mating calls but were most likely rustling sounds from carabid beetles. Only less than 1% of all ground attacks were initiated in response to mating calls of insects. However, in those cases, the mating calls were clearly detectable, allowing for species-level identification of the attacked prey. All these attacks on ground dwelling prey in response to mating calls were targeted at bush-crickets. Interestingly, however, the only bush-cricket species attacked by *M. myotis* was *Ephippiger ephippiger* despite the clear presence of other species such as *Tettigonia viridissima* and *Ruspolia nitidula* in the acoustic perceptual field of the tagged bats. This suggests that the acoustic cues provided by the mating songs of the bush-crickets contain information that bats can use to assess and discriminate their prey, and that the bats use this information and actively select their targeted prey species.

Furthermore, the integration of acoustic data with accelerometer and magnetometer measurements revealed that upon detecting the mating song of a bush-cricket, the tagged bats adjusted both their flight direction and speed towards the source of the song. This provides compelling evidence that wild bats actively utilise the courtship songs of bush-crickets as a means to track and successfully capture these singing insects. This finding represents a significant milestone, as it is the first direct empirical confirmation of such behaviour within the predator-prey relationship between bats and bush-crickets.

Objective 3

The final objective of this dissertation was to investigate the behavioural flexibility, external factors, and intrinsic influences on the sensory and behavioural defence mechanisms of bush-crickets against eavesdropping bats. This objective was accomplished by conducting behavioural experiments in controlled laboratory conditions. The experiment aimed to investigate whether the anti-predator behaviour of bush-crickets changes with the level of perceived predation risk and over the lifespan of the insects. For that purpose, eleven male adult *Tettigonia viridissima* were individually tested twice in their life in a dark flight room lined with anechoic material. Using a calibrated ultrasonic loudspeaker, the bush-crickets were presented with six experimental treatments consisting of four test-treatments that simulated four increasing levels of predation threat by echolocating bats and two control-treatments. The test-stimulus mimicked the echolocation calls of the lesser mouse-eared bat, M. blythii, and increased in sound pressure levels, to simulate increasing predation threat.

All bush-crickets stopped singing in response to high and moderate predation threat, but less so to low and very low predation threat. Both young and old bush-crickets paused their song for longer with increasing predation threat, with older males pausing for a shorter duration than younger males. Furthermore, in response to the very low predation threat and the cricket control sound, most or all males resumed singing before the end of the stimulus, indicating that they perceived these stimuli as less dangerous.

My results suggest that advertising male great green bush-crickets adjust their anti-predator behaviour (stopping their courtship song) to the level of predation threat, pausing more often and for longer periods when the level of predation threat was high, and to their age, with older males pausing for shorter periods than younger males. Older males therefore likely improve their likelihood to mate at the cost of being more susceptible to bat predation. We suggest that over their entire reproductive lifetime, the males trade-off the risk of losing out on mating opportunities against the risk of falling prey to eavesdropping bats, to optimise their overall reproductive success.

Main Conclusions

This dissertation has provided a comprehensive exploration of the predator-prey interactions between the greater and lesser mouse-eared bats (*M. myotis* and *M. blythii*) and their prey, bush-crickets (Orthoptera, Tettigoniidae). Through a combination of field observations, behavioural experiments, and molecular data, several key findings have emerged, contributing to our understanding of the sensory and behavioural dynamics within this complex predator-prey system. These key findings are consolidated in following nine main conclusions, which cover the areas of the dietary diversity of *M. myotis* and *M. blythii*, the foraging strategies of the *M. myotis*, the utilisation of acoustic cues in bush-crickets' mating calls by bats, and the defence mechanisms of bush-crickets against eavesdropping bats. Altogether, they illuminate intricate dynamics within the predator-prey system of these species.

Taxonomic diversity in the diet of the greater and the lesser mouse-eared bats

1. The faecal sample analysis of *M. myotis* and *M. blythii* revealed 1018 BIN species from various taxonomic groups, highlighting the taxonomic diversity within the sampled ecosystem. We have also revealed the presence of various ecto- and endo-parasites, as well as other associated species such as molluscs and worms. Using metabarcoding as a powerful tool, we provided an in-depth dataset to study the complex ecological network involving predators, prey, and their associated organisms within the ecosystems.

Foraging strategies of the greater mouse-eared bat

2. Greater mouse-eared bats (*M. myotis*) exhibit flexibility in their foraging strategies, utilising both ground gleaning and aerial hawking. Despite their specialisation for ground gleaning, they frequently engage in aerial hawking as a backup strategy, capturing smaller prey on-the-wing. Their foraging behaviour is characterized by adaptive prey switching in response to environmental dynamics. When changes in the environment reduce the profitability of ground prey, the bats switch to aerial hunting to ensure an adequate energy intake. This dynamic prey switching behaviour plays a crucial role in meeting their energy requirements, even for a specialised predator like the greater mouse-eared bat.

3. This adaptability allows them to maintain consistently high success rates and prey capture rates, irrespective of the habitat. Thus, aerial hawking may be a more widespread foraging strategy among gleaning bat species than previously believed. The reliability and

effectiveness of hawking as a backup strategy underscore its potential significance in securing food resources within fluctuating environments. This adaptive behaviour likely evolved to tap into unpredictable and ephemeral food sources in open spaces, providing an additional means of energy intake and enhancing resilience to changing conditions.

4. While greater mouse-eared bats do switch their primary prey type, their foraging predominantly relies on ground gleaning, with a focus on larger, energy-rich prey items. Despite experiencing relatively lower rates of prey capture and encountering failures on the ground, the overall profitability of such prey remains high due to their substantial size. This emphasizes the pivotal role of prey availability, size, and hunting success as key factors influencing the foraging decisions of wild bats.

Acoustic cues in the mating calls of the bush-crickets

5. By using biologging we have provided direct evidence that eavesdropping bats adjust their flight direction and speed towards the singing bush-cricket once its mating song becomes detectable. This confirms the hypothesis that bats utilize the courtship songs of bush-crickets to track and capture singing insects. Interestingly, the bats exhibit selectivity in the bush-cricket species they hunted, attacking only one of at least three different species in their acoustic perceptual field. This indicates that the mating songs of different species contain specific information that the mouse-eared bats use to assess whether to attack the prey or not.

6. Ground attacks by the bats are primarily triggered by rustling sounds from carabid beetles, and not by mating calls. Only a small percentage of ground attacks were initiated by mating calls of insects.

Defence mechanisms of bush-crickets against eavesdropping bats

7. Bush-crickets first exhibit an initial exploratory song pausing behaviour in response to supra-threshold sounds, followed by an adaptive extension of pause duration as a response to actual predation threats. This behaviour enables them to assess the threat level of the sound they receive, weigh the estimated risk of predation against the significance of courtship, and decide whether and for how long to utilize anti-predatory song cessation.

8. The cessation of singing in bush-crickets is a threat level-sensitive anti-predator response that rapidly evaluates and adapts to external sensory cues. This response serves as a means of predator avoidance and allows the bush-crickets to adjust their behaviour based on the perceived threat level.

9. Ageing male bush-crickets exhibit a higher tolerance for predation threats during courtship, supporting the terminal investment hypothesis. This suggests that older individuals allocate more effort into attracting mating partners and less into avoiding predation, to prioritise maximising reproductive fitness towards the end of their expected lifetime.

Together these findings contribute valuable insights into the intricate relationships between predators and prey, and provide a foundation for future studies of these complex interactions. By deepening our understanding of the mechanisms that underlie predator-prey interactions and the resulting food webs, we can better inform management strategies and mitigate potential anthropogenic impacts on biodiversity. Ultimately, this research serves as a steppingstone for future investigations, inspiring further exploration into the captivating world of sound-based predator-prey interactions and its implications for the preservation of our natural world.

V. Scientific contributions

Theoretical contributions

1. The taxonomic diversity in the diet of the greater and lesser mouse-eared bats in Bulgaria has been determined. For the first time molecular metabarcoding analysis was used to analyse the diet of the lesser mouse-eared bat.

2. The two main hunting strategies of the greater mouse-eared bat (aerial capture and ground capture) have been identified, and the number of attacks and the hunting success of the bats for both strategies have been measured and summarised.

3. The influence of habitat on the hunting success of the greater mouse-eared bat has been determined.

4. The size, quantity, and energy value of insects consumed by an individual bat per night have been identified.

5. Direct evidence has been collected that, during hunting, the greater mouse-eared bats utilize the mating calls of bushcrickets to locate and capture singing insects, supporting the hypothesis that gleaning bats eavesdrop on the mating signals of their prey.

6. The effects of age and threat level on antipredator behaviour during mate attraction by bushcrickets have been determined.

7. Evidence linking the Threat Sensitivity Hypothesis and the Terminal Investment Hypothesis has been presented, illustrating that prey species balance the costs and benefits of reproduction and survival throughout their lifetime.

Applied Contributions

8. An Open Access data paper incorporating the metabarcoding and bioinformatic analysis of the 113 faecal samples from *Myotis myotis* and *Myotis blythii* as well the raw molecular data has been published ensuring reproducibility and reusability of the data.

VI. Acknowledgments

First and foremost, I want to express my greatest and most sincere gratitude to my supervisor, Dr. habil. Holger R. Goerlitz, the person who guided me on the long journey to becoming a scientist. Thank you for teaching me how to think critically, to delve through layers of information until it actually makes sense, and to pay attention to the small but crucial details. Thank you for helping me structure the hurricane of racing thoughts and ideas in my head, for granting me research freedom and for always supporting my wild ideas and hypotheses. Thank you for believing in me and for always treating me as an equal. We might not have always made the best decisions, nor did we always manage to communicate flawlessly, but we did finish it. And I am so incredibly proud of what you and I, as a team, achieved together. Thank you for being my Doktorvater!

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The roots of this work go far back in time, in my childhood and the bedtime stories of my grandfather. Stories about scientific symposiums, pirates, flying chairs and brave engineers. In that magic land of wanders and knowledge inhabited by fairy-tale creatures, nature, laughter and joy that my mother created for us. Back to the small rooftop room in our summer villa where my sister would read to me the books of Gerald Durrell because I couldn't read myself. It started there and continued all the way to the present day because of the unwavering support of my family: my mother Elisabeth, my father Yuri, my sister Radosveta, my brother-in-law Dilyan, my nephews Maria and Kiril, my grandmothers Radka and Lilli, and my grandfathers Kiril and Antonii.

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We need to change that.

VII. Dissertation publications and conference attendance

Publications

Stidsholt L., **Hubancheva A.**, Greif S., Goerlitz H.R., Johnson M., Yovel Y., Madsen P.T. (2023) Echolocating bats prefer a high risk-high gain foraging strategy to increase prey profitability. *eLife* 12: e84190. IF: 8.71, Q1
https://doi.org/10.7554/eLife.84190
Contributions: Hubanchvea A. – Data curation, Formal analysis, Visualization, Methodology, Project administration, Writing review and editing. The paper is not part of another PhD Thesis.

Hubancheva, A., Bozicevic, V., Moriniere, J. and Goerlitz, H. (2023) DNA metabarcoding data from faecal samples of the lesser (*Myotis blythii*) and the greater (*Myotis myotis*) mouse-eared bats from Bulgaria. *Metabarcoding and Metagenomics*, 7: e106844. IS: 3.24, Q1
https://doi.org/10.3897/mbmg.7.106844
Contributions: Hubancheva A. – Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Project administration, Validation, Visualization, Writing original draft, Writing review & editing. The paper is not part of another PhD Thesis.

Preprints

Hubancheva, A., Senderov, V., Nowotny, M., Schöneich, S. and Goerlitz, H.R. (2023) Bush-crickets show lifelong flexibility in courtship signals to match predation threat. *Authorea*. August 16, 2023.
https://doi.org/10.22541/au.165167283.37444741/v3

Conference attendance

16th Invertebrate Sound and Vibration International Meeting, Gissen, Germany, 2017. “Acoustic response of European bushcrickets to bat echolocation calls” Poster presentation

Bats of Eastern Europe: challenges for conservation, Yerevan, Armenia, 2018. “Trophic niche differentiation of the sibling bat species Myotis myotis and Myotis blythii in Bulgaria” Oral presentation

German Bat Research Meeting, Berelin, Germany, 2018. “Acoustic response of European bushcrickets to bat echolocation calls” Poster

13th International Congress of Orthopterology, Agadir, Morocco, 2019. “Bushcrickets adapt predator avoidance behavior to predation threat levels and age” Poster presentation

15th Annual Meeting of the Ethological Society, Tübingen, Germany, 2020. “Anti-predator behavior in male bushcrickets is adapted to threat and age to optimize reproduction” Poster presentation

1st World Bat Twitter Conference, online, 2020, “The acoustic war between bats and katydids” Best Presentation Award

58th Annual Conference of the Animal Behavior Society, online, 2021. “A mating call that protects from predators” Oral presentation. Session Chair

Active Sensing: From Animals to Robots, 2023, Weizmann Institute of Science, Israel. “Mating calls of prey interfere with the sensory systems of bats” Oral presentation

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Декларация за оригиналност и достоверност

От Антония Юриева Хубанчева

Във връзка с провеждането на процедура за защита на дисертация за придобиване на ОНС „Доктор“ в Института по биоразнообразие и екосистемни изследвания – БАН, еднозначно декларирам:

1. Резултатите, обсъжданията и изводите в научната продукция, които предоставям в процедурата, са оригинални и не са заимствани без цитиране от изследвания и публикации, в които нямам участие.

2. Представената от мен информация във вид на копия на документи и публикации, лично съставени справки и др. съответства на обективната истина.

13.06.2023 г.

гр. София

Декларатор:

/Антония Хубанчева/