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

A PhD thesis by Ms. Antoniya Hubancheva

Review written by Hannah ter Hofstede, Assistant Professor, University of Windsor, Canada.

This review contains eight components, as requested by the University.

1. Dissertation Overview

The overview is intended to provide an evaluation of the structure and scope of the dissertation. I would first like to say that I really enjoyed reading this thesis. It consists of three chapters that address fundamental ecological questions about animal behaviour from the perspectives of both predators and prey. Specifically, it investigates gleaning bats that eavesdrop on the acoustic cues and signals produced by insect prey as a study system for testing hypotheses about how animals optimize foraging, mating, and antipredator defence decisions.

The first chapter is not hypothesis-driven, but instead provides a very detailed look at the diet of two predators in this predator-prey study system. This is a valuable contribution, since it provides the background information needed to support the focus on the specific species in chapters 2 and 3. It supports previous studies (using different methods) that the two bat species show niche partitioning with respect to the prey that they capture through gleaning, with *Myotis myotis* primarily gleaning beetles and *Myotis blythii* primarily gleaning katydids. It specifically identified six genera of katydids that form part of *M. blythii*'s diet, with *Tettigonia* spp. being the most common. Unlike previous studies, it also reveals overlap in the prey that would be captured using aerial hawking by both species, such as Diptera and at least some of the Lepidoptera. This first chapter therefore has two main functions. First, it identifies the prey sources that are either partitioned or shared by these two bat species. Second, it sets the stage for Chapter 2, which investigates how and why *M. myotis* switch between the two foraging strategies of gleaning and aerial hawking, and Chapter 3, which investigates how one identified

major prey species (*Tettigonia viridissima*) of one of these bat species (*M. blythii*) makes decisions about advertising for mates given the risk of attracting bat predators.

The second chapter investigates how *M. myotis* uses two different foraging strategies, gleaning and aerial hawking, and whether the choice of foraging strategy is related to energetic needs, habitat or other factors. The data clearly show that *M. myotis* have a higher success rate when aerial hawking, but they invest more effort in gleaning because the prey are larger and thus are energetically more profitable. Habitat did not affect the number of gleaning attacks on prey, although the bats were more successful in their attacks in fields than in forest. Finally, environmental conditions appeared to influence the foraging strategy used by the bats because bats foraging on the same night were more likely to use the same strategies. This study tested fundamental hypotheses about optimal foraging behaviour in animals and found compelling evidence to support the conclusions.

The third chapter tests the terminal investment hypothesis in the katydid *T. viridissima*, a major prey item in the diet of *M. blythii*. These katydids pause or cease singing when they hear echolocation calls, but how this anti-predator behaviour changes over an animal's lifetime has not been previously examined. Using playback experiments, this chapter shows that both young and old katydids modify their response to bat calls based on the risk of attack, but old males paused singing for much shorter time periods than young males. This result shows that these katydids behave in a way that optimizes their probability of attracting a mate while minimizing their risk of predation, and that they are willing to assume greater risk to attract mates later in life when the probability of survival and finding a receptive mate are lower.

Altogether, these three chapters provide a very nice examination of the predator-prey relationship from both perspectives. I was a bit confused, however, by the introductory and concluding components of the thesis. In section II "Aim and objectives," objectives 1 and 3 clearly correspond with the content of Chapters 1 and 3, respectively. Objective 2 is to "investigate the acoustic cues in the mating calls that are exploited by bats for locating singing bush-crickets", but this does not align well with the content of Chapter 2 or the other chapters. Chapter 2 investigates the foraging behaviour of *M. myotis*, which is thought to rely mainly on the rustling noises that insects produce when moving through their environment. Video 2 is a

beautiful recording of a bat capturing a singing katydid, but I do not see other references to katydid song in this paper. In section IV, "Summary and main conclusions," the results of both chapter 1 and 2 are summarized under the heading of Objective 1, and results that I did not find in the chapters are given under Objective 2 (<1% of ground attacks by *M. myotis* were in response to katydid song, and this bat species responded to the song of one katydid species and ignored the songs of other katydid species; p 66 and 70). In addition, the acoustic properties of these katydid songs were not described, as I was expecting based on the wording of Objective 2. Finally, both Chapter 2 and 3 mention supplemental material that did not appear to be included in the thesis. If the results described in the summary and conclusion section are in these supplemental materials, it would be helpful to include them as well.

2. Literature Review

Antoniya has done an excellent job of citing the studies of greatest relevance to her work and demonstrating her knowledge of the most recent literature. I believe that her summaries of the literature on each topic demonstrate a strong understanding of the theoretical underpinnings of her research and their significance in a broader context.

3. Methodology

The diversity of methods used to test hypotheses for the studies in this thesis is one of its major strengths. Approaches include diet analysis using DNA metabarcording, using tags on wild bats (both in captivity and in the field) to gain data on habitat use and foraging activity, audio and video recordings of behaviour, and playback experiments. I am not qualified to assess the molecular methods used in chapters 1 and 2, but they are like those described in other studies and provide very detailed results. In chapter 2, the use of chewing sounds from an onboard microphone to assess prey capture success and size was a fantastic idea. I was also extremely impressed with the sample size of tagged bats and the time and effort that went into ground-truthing these data with animals in the lab. The GPS tags also provided an additional level of data that was very valuable. In chapter 3, playback experiments were used to assess katydid

responses to acoustic stimuli. I personally know the patience that is needed to conduct these kinds of experiments, and I think the methods were ideal for this study.

4. Results and Interpretations

In all three chapters, the results provide a significant increase in our knowledge of these animals. The interpretation of the results are all appropriate and logically follow the data that are presented in the excellent figures. I found the Krona charts to be a particularly effective way to communicate the diet analysis results. I discuss the specific significance of the results in the next section.

5. Scientific Contributions

All three chapters provide original scientific contributions that will have an impact on understanding predator-prey relationships in the wild. Looking at the interactions from both the predator and prey's perspective provides a balanced approach.

Chapter 1: The very detailed dietary analysis of *M. myotis* and *M. blythii* provides both support for previous studies and raises new questions. One of those new questions was how these bats use two different foraging strategies, which was addressed in Chapter 2. I found the difference in the Orthopteran prey revealed by the metabarcoding study of Chapter 1 fascinating. Specifically, the bat species that relies on prey rustling noises (*M. myotis*) had many crickets in their diet, whereas the bat species that relies on prey acoustic signals (*M. blythii*) had almost none, despite crickets producing loud and continuous nocturnal song. I am curious to ask Antoniya if she has ideas about the factors that might contribute to this pattern.

Chapter 2: This chapter contributes important new information on how and why gleaning bats might continue to use aerial hawking as a feeding strategy. Many studies have now shown that gleaning bats will also aerially hawk prey, but this is the first that I am aware of to investigate the relative time spent in each strategy in the wild. The results show that the bats prioritize the higher risk gleaning strategy because of the high energetic reward of the prey, and that the lower success rate of gleaning might be exacerbated by yet to be identified environmental factors. The authors suggest the logical hypothesis that unpredictable gleaning

conditions could have selected for continued reliance on aerial hawking as a secondary, or at times primary, feeding strategy in these bats, despite their morphological adaptations for gleaning.

Chapter 3: The combined approach of testing whether insects show graded responses to predator cues depending on threat level with the potential changes with age is an original and important contribution to the literature. The results justified this approach since they show that males adjust their response to risk as their reproductive potential decreases. I think the discussion of how the control "cricket" sound revealed the importance of exploratory pauses in singing to assess predator threat was a particularly insightful and interesting contribution.

6. Quality of Research Papers

Two of the chapters in the thesis have already been published in peer-reviewed journals and have therefore been through a rigorous external review process. The first chapter is published in a specialized journal (*Metabarcoding and Metagenomics*) that is most appropriate for the subject matter. The second chapter is published in a high-ranking journal (*eLife*) that has a broad readership across the life sciences. This paper will reach a large audience interested in the general ecological question of how animals optimize foraging decisions. The third chapter has not yet been published, but it is currently under review. I believe the study will also be of broad interest to behavioural ecologists and I am sure that it will be published soon.

7. Candidate's Involvement

The candidate is first or second author on the papers comprising the thesis. Each chapter provides a detailed outline of her contributions. Her contributions to all chapters are fundamental, extensive and significant, including conceptualization of projects, major contributions of data collection and analysis, and writing of the manuscripts. These descriptions clearly show that Antoniya's individual contributions are the driving force behind the combined scientific value of the thesis.

8. Recommendation

I recommend awarding the scientific and educational degree "doctor" to Antoniya Hubancheva for her thesis. I warmly congratulate her on her scientific achievements.