

How Individual Variation Shapes Ecological Niches in Two Insectivorous Bat Species

Corresponding Author: Mr Markus Milchram

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Summary

This article assesses variation in behaviour across time and space and in diets of two co-occurring bat species in Eastern Austria. The authors use state of the art techniques to describe differences between species, between individuals and within-individuals. More specifically, they employed a high-throughput automated tracking system to track movement of 58 individuals, metabarcoding analysis to describe bats' diets, and a thoughtful statistical framework to include uncertainties and analyse the datasets. I acknowledge the huge effort of the authors to collect and analyse the data, and the accomplishment in detailing the behaviour of two co-occurring bat species. The study is relevant for the field as similar assessments are few, and the manuscript is well written and pleasant to read.

I have some concerns about potential speculative propositions made by the authors to both justify the study and discuss the results. I suggest that the authors address this by both reviewing some of these propositions and clarifying methods. I have detailed below my main concerns and questions.

1. The authors built the text around the proposition that differences between and within species are mechanisms that enable coexistence of populations, and that they likely facilitated the range expansion of Kuhl's pipistrelle bats. However, the results don't show enough information for this conclusion, as we don't know other factors that may be enabling or limiting range expansion and coexistence. For example, there is no information about temporal changes in resources, land use and physical attributes, availability and competition for rooster sites, and interaction between species. Besides, the results show that there are some differences between the species and individuals, but also show that the niche overlaps are quite large. I suggest that the authors review the text, mainly the abstract, introduction and discussions, to make clearer for the reader that the results don't explain the range expansion of Kuhl's pipistrelle.

2. The authors analysed movement data mostly of female *P. kuhlii* and male *P. nathusii*. I'd like to know why they believe that the differences between species are not caused by sex specific behaviours. In line 184, for example, the authors mention that "female bats, especially during lactation, are known to return frequently to their roosts at night". Other studies, however, show that sex is not the most important trait shaping movement behaviours for some bat species in the tropics, such as Kerches-Rogeri et al. 2021 (Movement Ecology).

3. In line with the comments above, I suggest the authors give more information about the study system: the biology of the bat species, the study site landscape, and the study site biodiversity. I trust this will help the reader to understand the implication of the results. More specifically, it would be helpful to know differences in behaviour or morphology between sexes, if the species show territorialism, if they show some specific behaviour to prey on insects, if other studies have observed changes to local bats populations. On the study site, I'd like to know more about the relief of the site – and if it may have affected the transmitters detection – and see a map of the classified landscape showing rooster locations and the position of the receiver stations. I believe it's also relevant to know if there are other insectivorous bat species that may be competing with the focus species.

4. Since the methods are very complex, I suggest the authors illustrate them in a figure or illustrated flowchart. This could be added to the main text or the supporting material. It would be interesting to see the methodological steps, input data and rational of decisions.

5. I'd like to know how the guano sample collection was distributed on time and space, and which steps were followed to ensure they would reflect species diets. Samples collected in very different times, for example, can reflect temporal variation in resources availability.

6. I assume that different habitats offer different insects for the bats. Are you able to notice any relationship between the diet compositions and the different uses of habitats? Are the diet compositions reflecting the different uses of the habitats?

7. The accuracy of the tracking system (median of 294 m [IQR 153-756]) looks low to me if compared to other studies using the same methods. Gottwald et al. 2019, for example, was cited by the authors and estimated smaller errors (linear error of 21 m within a distance ranging from 65 m to 190 m). I'd like to know why this may have happened, and what are the implications of using a landscape map with higher resolutions (100 m). I can see that the authors followed several steps to include uncertainty into the models, but it's not clear what are the consequences of the uncertainty for the results and discussion.

Line-specific comments

Line 206 – Please rewrite to describe which landscape changes are expected.

Line 272 – “nine bats simultaneously”: line 87 says “up to eight individuals tracked simultaneously”

Line 347 – Please add units to margin and window sizes, or the equivalent in units that are usual to describe bats' movements.

Reviewer #2

(Remarks to the Author)

In this manuscript, Milchram et al. investigated niche partitioning at the species, between-individual, and within-individual levels in two bat species using radio-tracking and metabarcoding methods. They found substantial variation in habitat selection at the individual level. More specifically, one species exhibited pronounced between-individual variation, while the other showed greater within-individual variation, potentially explaining its ongoing range expansion observed in Austria and other regions.

I find this to be an interesting and well-written study in the field of community ecology, with a clear statement of goals and well-supported conclusions. I found the discussion especially clear and informative. Yet, I have some minor comments regarding other aspects of the manuscript.

Abstract

1)L30: Please specify the method used to obtain the spatio-temporal habitat selection data (i.e., radio-telemetry), as this is not entirely clear.

2)L35: Is *P. kuhlii* commonly described as an 'invading' species elsewhere? I would consider changing this term.

Introduction

3)L48: I suggest rephrasing to something like 'For instance, due to climate change...' to reduce the repetition of 'global change' in the introduction, this is more specific to climate change too.

4)L52: Could you briefly summarize how these variations have influenced range expansion, as reported in previous studies?

5)L61-63: I am not fully convinced by the conclusion of this paragraph; the conclusions will be quite specific to bats, given their high mobility and mammalian behaviour.

Results

6)L81: Is there a specific reason for the observed capture disparity between sexes within species?

7)L88-91: This content would be more appropriately placed in the Methods section.

8)L104-106: Would it be possible to include a map in the appendix? This could provide a clearer picture of the known roost locations, habitat distribution, and distances. It would also help assess the accuracy of the activity classification used.

9)Fig 1: *P. kuhlii* is shown in a pink circle, which is a different color from the lines.

10)Fig2: The two graphs at the top are a bit confusing. I would suggest simply labeling them as 'between-individual' and 'within-individual' at the top for clarity.

11)L135: Except for vineyards too, right? It is difficult to determine from the figures; it is not really clear for forests either.

Methods

1)L234: Did you detect a year effect in your analysis?

2)L392: “following”?

3)L404: Why did you use two different kits?

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)

All the comments have been addressed very carefully, and the new version of the manuscript appears to be of excellent

quality.

I have only one minor suggestion:

Line 262: To avoid the repetition that *P. nathusii* raises two pups, I would suggest reformulating to: "As for *P. nathusii*, *P. kuhlii* typically raises two pups."

Potential issue with PDF conversion:

Figure 2: There appears to be a font issue in the titles "between ..." and "within-...".

Figure S1: I cannot see the colors in the landscape legends or the symbols for the receiver station 2022.

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Reviewer 1:

This article assesses variation in behaviour across time and space and in diets of two co-occurring bat species in Eastern Austria. The authors use state of the art techniques to describe differences between species, between individuals and within-individuals. More specifically, they employed a high-throughput automated tracking system to track movement of 58 individuals, metabarcoding analysis to describe bats' diets, and a thoughtful statistical framework to include uncertainties and analyse the datasets. I acknowledge the huge effort of the authors to collect and analyse the data, and the accomplishment in detailing the behaviour of two co-occurring bat species. The study is relevant for the field as similar assessments are few, and the manuscript is well written and pleasant to read. I have some concerns about potential speculative propositions made by the authors to both justify the study and discuss the results. I suggest that the authors address this by both reviewing some of these propositions and clarifying methods. I have detailed below my main concerns and questions.

Response

We thank the reviewer for the positive feedback and the acknowledgement of our statistical framework. In response to the reviewers, we clarified the text where needed. As requested, we included a map of the study region, added a flowchart of the methods and answered questions on sex and guano sampling. We also clarified parts of the methods and responded to minor changes.

1. The authors built the text around the proposition that differences between and within species are mechanisms that enable coexistence of populations, and that they likely facilitated the range expansion of Kuhl's pipistrelle bats. However, the results don't show enough information for this conclusion, as we don't know other factors that may be enabling or limiting range expansion and coexistence. For example, there is no information about temporal changes in resources, land use and physical attributes, availability and competition for rooster sites, and interaction between species. Besides, the results show that there are some differences between the species and individuals, but also show that the niche overlaps are quite large. I suggest that the authors review the text, mainly the abstract, introduction and discussions, to make clearer for the reader that the results don't explain the range expansion of Kuhl's pipistrelle.

Response

*We agree that some of the propositions we made might not be clearly stated as being speculative, and we agree that there are other factors promoting the range expansion of *Pipistrellus kuhlii* (see Ancillotto et al. 2015 for details). As suggested, we weakened some statements where we agree that the choice of words does not meet our careful intentions. Specifically, we restructured the last sentence of the abstract (lines 33-36) and changed "facilitate" to "contribute" in line 220. In lines 221-222, the sentence "Although we lack data on other potentially influential factors, such as temporal changes in resources or land use" was added to clarify that there are other factors as well which are important to consider when speculating about the role of within-individual variation in the range-expanding *P. kuhlii*.*

However, we disagree with the comment that we proposed that “differences between and within species are mechanisms that enable coexistence of populations”. We stated that individual variation plays an important role in coexistence studies (lines 42 – 58), but whether they enable or hinder coexistence is not fully resolved yet (192 - 208).

We think that the framework we use to present our data (modified communities due to changes in species distribution) is important to justify our work. How niche partitioning can stabilise communities, which are reshaped during climate change, is a crucial question in ecological research (e.g. Audusseau et al. 2016 J. Biogeogr., González-Megías et al. 2008 Glob. Change Biol., Devictor et al. 2012 Nat. Clim. Change). Although we do not offer a complete resolution to this question, our emphasis on the role of individual variation in niche partitioning and our demonstration of differences in individual variation between species provide valuable insights.

*Additionally, within-individual variation in morphology and physiology has been shown to contribute to range expansion in invasive species (Davidson et al. 2011, Knop et al. 2012). Considering *Pipistrellus kuhlii*'s extensive range expansion and the apparent flexibility of individual bats in habitat use, we propose that this variation likely plays a role in its success.*

2. The authors analysed movement data mostly of female *P. kuhlii* and male *P. nathusii*. I'd like to know why they believe that the differences between species are not caused by sex specific behaviours. In line 184, for example, the authors mention that “female bats, especially during lactation, are known to return frequently to their roosts at night”. Other studies, however, show that sex is not the most important trait shaping movement behaviours for some bat species in the tropics, such as Kerches-Rogeri et al. 2021 (Movement Ecology).

Response

*We recognise the potential influence of sex-specific behaviours on our findings. As mentioned in lines 158-159, the greater between-individual variance observed in *P. nathusii* is likely attributed to the male-biased sex ratio in our study area. Additionally, as the reviewer noted, lines 183-184 state that lactating female bats frequently return to their roosts at night.*

*The sex bias in our study area is typical for *P. nathusii* in Central and Southern Europe, where males are present year-round, awaiting the arrival of females which raise their pups in Northern Europe and migrate south to mate and hibernate (Strelkov 2000 ; Pētersons 2004 *Myotis*, also known for other long-distance migrants such as *Nyctalus noctula* (Petit et al. 2001) or *Vespertilio murinus* (Lapini et al. 2015)). This information has been added to the Methods section (lines 246-268).*

We attempted to control for sex-specific differences in our analysis by including “sex” as a random factor. The resulting models did not substantially differ from those presented in the paper. However, the highly skewed sex ratios and consequent unbalanced sample sizes led to suboptimal model convergence, as indicated by our model diagnostics (i.e. potential scale reduction factor above 1.0.1). Therefore, we would not prefer to present these models in the paper. We suggest that future research applies our workflow to a system with more balanced sex ratios to investigate potential sex-related effects, a recommendation now included in the discussion (lines 209-215).

3. In line with the comments above, I suggest the authors give more information about the study system: the biology of the bat species, the study site landscape, and the study site biodiversity. I trust this will help the reader to understand the implication of the results. More specifically, it would be helpful to know differences in behaviour or morphology between sexes, if the species show territorialism, if they show some specific behaviour to prey on insects, if other studies have observed changes to local bats populations. On the study site, I'd like to know more about the relief of the site – and if it may have affected the transmitters detection – and see a map of the classified landscape showing rooster locations and the position of the receiver stations. I believe it's also relevant to know if there are other insectivorous bat species that may be competing with the focus species.

Response

We thank the reviewer for these suggestions. The section “Study species” was added to the methods, giving a short overview of the biology of the study species (246-268). We also included information about other bat species in the area (242-244).

Our study site is ideal for automated VHF-tracking because it is very flat. We specified this in the description of the study site (lines 236-237). Following the reviewer's suggestion, a map showing the landscape classification, roosting sites and receiver stations was added to the supplementary file (Fig. S1, referred to in line 239).

4. Since the methods are very complex, I suggest the authors illustrate them in a figure or illustrated flowchart. This could be added to the main text or the supporting material. It would be interesting to see the methodological steps, input data and rational of decisions.

Response

We included a flowchart to the supplementary file (Fig. S2) and referred to it in line 231.

5. I'd like to know how the guano sample collection was distributed on time and space, and which steps were followed to ensure they would reflect species diets. Samples collected in very different times, for example, can reflect temporal variation in resources availability.

Response

We agree that different sampling times can lead to temporal variations in resource availability. To minimise bias in our study, we sampled in three consecutive months (June, July and August) according to the tracking of bats. These months typically reflect the peak activity period of many ectotherms in temperate regions. Particularly, insect orders including Diptera, Coleoptera and Lepidoptera, favoured by various insectivorous bats, show peak activities during summer months (Tiede et al. 2020, Basic and Applied Ecology). In addition, we sampled in two consecutive years to minimise a potential sampling bias. While we carefully evaluated all metabarcoding results on a species level, we refrained from using species-level data for analyses. Instead, we analysed all data at the genus level, which helps to overcome species-specific sampling bias (lines 490-491).

To clarify the sampling scheme, we provide a column in supplementary table 1 detailing the temporal sampling of all metabarcoded bat guano samples. All guano was collected from captured bats within our study area. Given the short distance between sampling locations (maximum distance of 4 km), which is less than the bats' observed flight range and consistent with previous studies (e.g. Tiede et al. 2020), we do not expect the sampling location to significantly influence the dietary composition.

6. I assume that different habitats offer different insects for the bats. Are you able to notice any relationship between the diet compositions and the different uses of habitats? Are the diet compositions reflecting the different uses of the habitats?

Response

Parts of the varying uses of the habitats are likely explained by varying diet compositions in those areas. We stated that in the discussion (172-174). However, our analysis at the genus level limits a more detailed investigation of the habitat-prey relationship. Many insect genera contain multiple species with differing habitat preferences, and for many insects, specific habitat information is lacking. Further investigation of prey availability in different habitats (e.g., using insect traps) could be applied to explore this relationship further.

7. The accuracy of the tracking system (median of 294 m [IQR 153-756]) looks low to me if compared to other studies using the same methods. Gottwald et al. 2019, for example, was cited by the authors and estimated smaller errors (linear error of 21 m within a distance ranging from 65 m to 190 m). I'd like to know why this may have happened, and what are the implications of using a landscape map with higher resolutions (100 m). I can see that the authors followed several steps to include uncertainty into the models, but it's not clear what are the consequences of the uncertainty for the results and discussion.

Response

Deploying VHF-tracking systems involves a trade-off between accuracy and range: closer station spacing increases accuracy but can also increase the risk of bats leaving the tracking area. We opted for a less dense network covering a larger area, given known long-distance bat movements. For example, Gottwald et al. (Figure 4) used 200 m station spacing, whereas our average spacing was 965 m.

Additionally, the size of the error depends on the positions of the transmitters relative to the stations. Gottwald et al. used a testing scheme with ideal positions of tags relative to stations (about 45°, Fig. 2 in Gottwald et al.). Under such conditions, we also recorded errors of 21.5 m. However, when transmitters were positioned near 0° or 90°, substantially larger errors were observed, contributing to our reported median error of 294 m. This relatively coarse resolution is consistent with our 100 m × 100 m land cover map. Improved tracking precision would likely permit the analysis of smaller landscape elements, such as hedgerows and individual trees.

Line-specific comments

Line 206 – Please rewrite to describe which landscape changes are expected.

Response

Specified (lines 206-207)

Line 272 – “nine bats simultaneously”: line 87 says “up to eight individuals tracked simultaneously”

Response

Line 87 is correct, we changed that in line 309.

Line 347 – Please add units to margin and window sizes, or the equivalent in units that are usual to describe bats' movements.

Response

We added “locations” (384-385)

Reviewer #2 (Remarks to the Author):

In this manuscript, Milchram et al. investigated niche partitioning at the species, between-individual, and within-individual levels in two bat species using radio-tracking and metabarcoding methods. They found substantial variation in habitat selection at the individual level. More specifically, one species exhibited pronounced between-individual variation, while the other showed greater within-individual variation, potentially explaining its ongoing range expansion observed in Austria and other regions.

I find this to be an interesting and well-written study in the field of community ecology, with a clear statement of goals and well-supported conclusions. I found the discussion especially clear and informative. Yet, I have some minor comments regarding other aspects of the manuscript.

Response

We thank the reviewer for the feedback and the valuable comments. Suggestions were implemented and our response was added below.

Abstract

1)L30: Please specify the method used to obtain the spatio-temporal habitat selection data (i.e., radio-telemetry), as this is not entirely clear.

Response

We added "radio-tracked" (line 29)

2)L35: Is *P. kuhlii* commonly described as an 'invading' species elsewhere? I would consider changing this term.

Response

We removed the term (line 36), because we think there is no need for a specific term in this sentence.

Introduction

3)L48: I suggest rephrasing to something like 'For instance, due to climate change...' to reduce the repetition of 'global change' in the introduction, this is more specific to climate change too.

Response

Changed (line 48).

4)L52: Could you briefly summarize how these variations have influenced range expansion, as reported in previous studies?

Response

We added a specification of this statement (lines 53-56).

5)L61-63: I am not fully convinced by the conclusion of this paragraph; the conclusions will be quite specific to bats, given their high mobility and mammalian behaviour.

Response

The high mobility of bats, even exceeding that of many other mammals, combined with their strong dependence on temperature, suggests a potential for rapid responses to climate change. However, temperature optima and mobility are widespread in animals, although other species might react slower. Thus, our conclusions may be relevant to other species, although their responses are likely less pronounced.

Results

6)L81: Is there a specific reason for the observed capture disparity between sexes within species?

Response

*Sexual segregation is typical in many bat species, especially in migrating species such as *Pipistrellus nathusii* (Russ et al. 2023). We refer to the response to remark 2 of reviewer 1, where we answered this question in more detail. We also added a paragraph to the methods section (246-268) to give more information about the study species.*

7)L88-91: This content would be more appropriately placed in the Methods section.

Response

This information has been removed from the results section. A methodological flowchart (Fig. S2) in the supplementary material provides the reader sufficient context for understanding the results.

8)L104-106: Would it be possible to include a map in the appendix? This could provide a clearer picture of the known roost locations, habitat distribution, and distances. It would also help assess the accuracy of the activity classification used.

Response

We added a map of the study area to the supplementary file (Fig. S1)

9)Fig 1: *P. kuhlii* is shown in a pink circle, which is a different color from the lines.

Response

This error might have happened during the conversion to a .pdf. In the new version, the figures were implemented as .svg which should resolve this effect.

10)Fig2: The two graphs at the top are a bit confusing. I would suggest simply labeling them as 'between-individual' and 'within-individual' at the top for clarity.

Response

Corrected.

11)L135: Except for vineyards too, right? It is difficult to determine from the figures; it is not really clear for forests either.

Response

*Differences in vineyards were not significant (corrected in line 133). Because the confidence intervals (CIs) of *P. nathusii* do not overlap the posterior estimates of *P. kuhlii* in forests and arable land, we interpret these differences as significant.*

Methods

1)L234: Did you detect a year effect in your analysis?

Response

We corrected for annual and seasonal effects by implementing date as a random factor (420-421). Out of curiosity, we ran the model with year as a fixed factor but did not detect a significant year effect on the results.

2)L392: “following”?

Response

Following Housley et al. We added the authors in line 429.

3)L404: Why did you use two different kits?

Response

Thank you for the thorough review. We apologise for the error. Only one DNA isolation kit, the QIAamp PowerFecal Pro DNA Kit (Qiagen, Hilden, Germany), was used in this study. All samples in this study were processed using a uniform protocol with the QIAamp PowerFecal Pro DNA Kit. The Materials and Methods section has been revised accordingly (lines 438-444 and 463-467) to correct the previous mention of two kits, a remnant from an earlier draft.

Editor:

Reviewer #1, point #6: please add text to the discussion similar to your response in the rebuttal, to acknowledge limitation regarding habitat-prey relationship.

Response

We included the text accordingly in lines 178-184.

Please also add text about the tradeoff between accuracy-range to either the main text or supplementary material (e.g., supplementary note), related to your response to Reviewer #1, point #7.

Response

We included the text "Deploying VHF-tracking systems involves a trade-off between accuracy and range. Closer station spacing increases accuracy but may also increase the risk of bats leaving the tracking area. Considering the anticipated long-distance movements of bats, we decided on a less dense network that covers a larger area of approximately 85 km²." to Supplementary Note 1, last paragraph.

Reviewer 2:

Line 262: To avoid the repetition that *P. nathusii* raises two pups, I would suggest reformulating to: "As for *P. nathusii*, *P. kuhlii* typically raises two pups."

Response

Changed (Line 269).

Potential issue with PDF conversion:

Figure 2: There appears to be a font issue in the titles "between ..." and "within-...".

Figure S1: I cannot see the colors in the landscape legends or the symbols for the receiver station 2022.

Response

This is interesting. On our screens, we could not observe these effects – we hope this bug is only appearing on the reviewer's end.