

The manuscript “*Cities as parasitic amplifiers? Malaria prevalence and diversity along an urbanization gradient in great tits*” analyze the effect of the urbanization on the prevalence and diversity of avian malaria parasites in the great tit, through two methodological approaches or spatial scale: compares an urban environment (with 8 sites/replicas) vs. a non-urban environment (1 site only) and then, a gradient of urbanization within the urban environment. I find some problems or lack of clarity in this methodological part. It remains to be described how the data is distributed over the years, and there is a great imbalance between the number of samples from the urban and non-urban environments. At the same time, I believe that the heterogeneity of the urban environment must be considered in analyzes at the nest scale, including the site as a random variable through generalized linear mixed models, for example. The results of the models are not so conclusive (some have enormous confidence intervals, or include 0), to express it that way in their conclusions. You should be much more cautious when providing your conclusions. Although it is a very interesting and necessary work from the point of view of disease ecology and also challenging when studying the environment or context that is in constant change, there are some important methodological issues to review. A proposal or alternative to think about, could be to consider 3 environments: urban, semi-urban and rural or forest with a homogeneous or similar number of samples and explore in greater detail the characteristics of these environments and how the prevalence varies. The semi-urban environment could consider some of the sites on the periphery of the city and that gather a considerable number of samples.

Title: I suggest change the order of the last part of the title. *Cities as parasitic amplifiers? Malaria prevalence and diversity in great tits along an urbanization gradient.*

Add the scientific name of the great tit and it would also be appropriate to add in Montpellier, France or, at least, France.

Abstract

Line 40: I consider that it is a somewhat alarmist conclusion and due to the experimental design and/or methodological problems I would not have enough support to say this. Furthermore, it is not correct to use the concept of epidemic if we are talking about avian malaria. Try to be more cautious with the conclusions.

Keywords: I suggest adding France to the list.

INTRODUCTION

Line 74 and 81: Criticizes the binary perspective of urban vs. not urban but presents and analyzes its results in that same way.

Line 85: Move this sentence. The paragraph could start directly with... “Avian malaria parasites...”

Line 103-109: It's being repetitive and getting confusing. I suggest eliminating the first sentence and starting with the sentence that starts from “Specifically...” pointing out the 3 objectives.

Maybe it starts here with the paragraph and sentence on line 85.

Line 104: Spatial resolution is another thing; I imagine you mean to refer to a spatial scale. Modified.

METHODS

Line 128-129: Wouldn't it also be important to include variables relevant to the biology of the vectors? For example, distance to water bodies or presence and size of water bodies... Think about which variables could influence the ecology of malaria and be masked within the "urbanization" variable.

Line 141: The PCA is usually used when you want to summarize the number of variables, since there are many explanatory variables that are correlated with each other. On the one hand, 4 variables seems like a small number of explanatory variables to me (considering that then there is a glm with only 1 vs. Explanatory), and on the other hand it would be good to incorporate the graph to see how the variables relate to each other (although be in the supplementary material).

Line 154: Blood sample collection... How is the blood sample collection over the years? It would be necessary to incorporate a table specifying all sampling sites, type of habitat, year of sample collection, number of samples (nestlings and adults), etc.

Line 159-160: How many sites were from the urban environment and how many from the non-urban environment? Specify for both chicks and adults. This should be in the table requested in the previous point.

Line 214: Change resolution by spatial scale.

Line 222: Fix next by nest box.

RESULTS

Line 273-274: Careful. The value of the estimate and the CIs are very large. It may be because in the non-urban environment there are only 0.

Line 279: Close parentheses.

Line 288-289: Again, be careful with these results. The confidence interval includes 0 so it would not be significant.

DISCUSSION

Line 429: Surprisingly however, it would not be a good expression to start the paragraph. I would start directly with..."the diversity of Haemosporidian lineages

Correct Haomosporidian by Haemosporidian.

Be much more cautious with your conclusions throughout the discussion. Problems with experimental design and number of samples heterogeneity may be leading to erroneous results. In addition, there are many variables that can affect the host and the vectors that may be masked within the urbanization variable and were not taken into account.

Figure 1: Add in the regional scale that are shown the non-urban habitat (ROU) and the urban habitat (Montpellier).

Figure 5 and 6: Change location of figures to before discussion.

There are several spelling errors in the supplementary material. The word Plasmodium is misspelled several times. Review.