Prof. Dries Bonte and Dr. Silke Bauer

Editors of *Oikos*

Dear Prof. Bonte and Dr. Bauer,

We are submitting a **revised version** of manuscript **OIK-08071**, in which we have made changes in response to the comments received from the reviewers. We are grateful for the opportunity to revise and send you a new version.

Below we have pasted the reviewers’ comments, and inserted our RESPONSES after each comment, as well as indicated where in the text the relevant changes have been made.

We hope the manuscript is now acceptable for publication.

Sincerely,

Daniela de Angeli Dutra and co-authors

**EDITOR DR. BAUER:**

First, indeed migratory birds are often assumed to disperse parasites along their migration routes. However, migration can also be a mechanism of reducing parasite prevalence through migratory escape, separation, etc. I would like to see this mentioned in the ms and the results discussed also considering these counteracting mechanism. Although this is partly brought up in the discussion, I would expect this much earlier in the paper.

We now mention how migration can be a mechanism that may reduce as well as increase parasite infections in the introduction. L30-34. We also introduce the possibility of migration as a mechanism to escape from environments with greater risk of *Plasmodium* infections in the discussion. L327-328.

The presentation of all the models (and reports of their results) needs to be made much clearer and more concise. Please restructure the corresponding sections.

We realise the model presentation may have been a bit confusing and we have now restructured this section to make it as clear as possible.

I would also like to see effect sizes explicitly mentioned and explained in the results (e.g. how much larger is the geographic range of parasites that occur resident and migrants, etc.).

We now explicitly mention how much larger the geographical range was for lineages shared by resident and any migrant species compared to those found only in resident birds. L232.

Please also provide a table with bird species and their categorization as residents, partial or full migrants (perhaps in the supplement.

This was a good point. The table is now provided as supplementary table 2, and referred to in the first paragraph of the Methods section.

Minor comments

- L. 86 & 87 – “using” sounds a bit odd, perhaps infecting or found in might be more suitable

Modified accordingly. We replaced the word “using” with “infecting”

- L. 125 – please provide reference or link to WorldClim

Modified accordingly. The link is now given on line 125

- L. 145. Entire sentence seems redundant as same info given already in l. 143

We appreciate this comment and now realise the text was a bit confusing. However, the sentences are not redundant. In line 143 we explain we used only lineages present in resident species or lineages present in resident species plus another migratory status. In line 145, we specified that we discarded all the localities where lineages were present only in migrant species. We have now modified the text to make it clearer.

- L. 150. Change “..decided to build” to “built”

Modified accordingly. L150

- L. 155. Replace performed by run

Modified accordingly. L157

- L. 155. The second model seems redundant as you have already in the firs model a category for resident + partial/full migratory. Alternatively, this is contradicting your earlier statement (in first paragraph on this page).

We appreciate this comment and agree with the editor that our results may have seemed redundant, and that the use of two similar models can impair our interpretation of the results. However, the category resident+partial/full migratory does not illustrate the combination of all lineages present in residents plus any migrant, but only the lineages present in all three avian species categories. Thus, it does not lead to the same results. We have now modified the manuscript to make it clearer (L151-155).

- L. 162ff – why separate models and not parasite genus included as a factor?

We decided to run separate models for each parasite genus due to their different life cycles and biological characteristics, which could impact parasite dispersal, such as the use of completely different vectors and distinct levels of avian specificity (Valkiunas 2005).

- L. 175. Omit “bird” before prevalence

Modified accordingly. L177

- L. 176. Proportion of migr birds – you mean the number of individuals of mig species and not the proportion of mig species in the species pool at a locality, right? Please clarify.

Thank you for pointing out the text was confusing; it has now been modified accordingly. L177-178

- L. 178. Replace in by on

Modified accordingly. L180

- L. 212. Replace “classed” by categorized or classified

Modified accordingly. L214

- L .216. “only” sounds counterintuitive with 175 lineages. How many were there in total?

The total number of lineages was 675. We added this information again in the results section. L218

- L. 219. I guess “the” should be “that”?

Modified accordingly. L221

- L. 285. Partly redundant, please rephrase and/or merge sentences

Sentence has now been rephrased. L285-287.

- L. 306. Such pattern could also be found if effects of parasites (lineages) on birds differ. See general comment on migration as a mechanism to change prevalence

Good point, this additional viewpoint has now been added to the manuscript. L309-312

- Fig. 1 seems to be of relatively low quality - perhaps this resulted from the conversion but please check.

A new better-quality figure has been provided

- Fig. 3 is redundant, info already provided in Fig. 2. In Fig2 spell out x-axis labels (rather than providing them in legend)

As we explained in above, the results presented in the two figures are actually not redundant. Therefore, since they present different findings, we prefer to keep them both.

- Combine Figs. 4& 5 into one multi-panel figure

The two figures have now been combined as suggested.

- Fig. 6. Needs more explanation. I wasn’t sure which model results are shown here. Incidence rate ratios seem not to be explained in the text; % migrants, %migrant species and number of migrants – seem like correlated variables – please clarify

Thank you for pointing this out; both the figure and legend have now been modified.

**REVIEWER #1:**

This study is based on an impressive data from South America, consisting of blood samples from ~15,000 individuals (506 bird species / 156 localities), examined for presence and identification of haemosporidian parasites.

The first hypothesis asks whether “migratory birds spread parasite lineages along their migratory routes” which is also the title of the manuscript “Can migratory birds spread avian haemosporidian parasites?” This is a somewhat trivial question to ask given that many publications have shown that migrants and local residents are sharing haemosporidian parasites, and that migrants frequently carry parasites outside the typical transmission range of the parasite (providing potential, but not yet realized, spread to resident birds).

We feel that this question remains an important one. Firstly, not all studies have concluded that migratory birds disperse parasites. Secondly, answering this question is a necessary first step toward our second question, which the reviewer found much more interesting (see below). Our study adopts a two-pronged approach to look at the role of migratory hosts in dispersing parasites, and together our two questions provide a stronger test of the general migration-infection connection. Nevertheless, we have modified our title to shift the emphasis more toward the second question.

This hypothesis is tested by comparing the ranges of parasites found in both migrants and residents with parasites restricted to residents. I am not convinced that this a conclusive test of the hypothesis; if the former are found to have larger ranges (interpreted as caused by the spread of migrants) this could equally be explained by common parasites being more likely to infect both migrants and residents.

We understand the reviewer’s point of view and we agree that a greater geographical range could be explained by common parasites being more likely to infect resident and migrant hosts. However, it is exactly for that reason that we controlled for both parasite abundance (number of times each lineage was present) and generalism (number of avian species in which a lineage was found) in our analyses. See L161-163. Hence, we believe the greater geographical range observed in lineages shared by both resident and migrant species is due to migration dispersal.

The second hypothesis is of more general interest; is the spread of parasites by migrants of such magnitude that it has a measurable effect on the prevalence and richness of parasites in the host-parasite communities along the migration routes? I think the manuscript would be much stronger with a focus on this question. More details are also need on how you have calculated some of the key variables, like proportion of migrants in the community and species diversity.

We thank the reviewer for pointing out the originality of this question. As we argued above, we still think that keeping the focus of our article on both questions makes it a more comprehensive test of the migration–infection link. As suggested by the reviewer, how we calculated the proportion of migrant individuals in an area is now clearly mentioned and explained in L178-181, as well as the fact that when comparing community prevalence we evaluated all birds present – including migrants. We also explain that we accounted for phylogenetic influences in our analyses in L198.

In some places in the discussion, you are drawing too strong conclusions (demonstrating effects) as your findings are more correctly statistical associations between variables.

We have modified the manuscript to address this issue as suggested below, to tone down our wording regarding effects versus associations.

Detailed comments

Line 37. Not clear what you mean by “act as an environmental filter to new species colonization”

Thank you for pointing out the confusion; we have now modified the manuscript. L37-39.

Lines 165-170. When you tested whether prevalence is higher in localities with more migrants, I wonder whether you only included resident species in the analyses. It is not clearly stated but I feel that this is how it should be done given that you want to know whether migrants have any impact on the local parasite community.

When we analysed community prevalence, we used the whole avian community including resident and migrants. We understand the reviewer’s point of view, however, we believe that since migrants are also part of the local avian community and most species are partial migrants (and therefore we cannot distinguish if an individual bird migrates or not) we decided to consider all avian species in our analyses.

Lines 180-181. “include only species with 10 or more birds..” In total or minimum 10 / locality?

We apologise for not making this clear and we have now modified the text. In this case, we used only species with a minimum of 10 bird individuals per species in each locality where that bird species occurred. See L184

Line 235. “Proportion of migratory birds in the local avian community”. I could not find how you obtained this estimate. Is it the proportion of migrants among the captured birds? If this is the case, I wonder how well this would correspond to the actual “Proportion of migratory birds in the local avian community” as there are many potential biases (e.g. migrants may be more or less easy to catch depending on whether they are ground, canopy or edge foragers). Also, how was the catching at the 155 localities timed relative to the expected presence of migrants?

We estimated the proportion of migrant individuals using the data on captured birds. Naturally, we understand the reviewer’s concern, however, since we use a large dataset and the capture method was similar in all 63 localities, we believe the potential bias should not be impacting our results. Indeed, we observe for example, a small proportion of migrants in regions where this was already expected, such as the amazon region where most birds are residents. Besides, mist nets capture both migratory and resident birds with the same probability in the strata where they are located from 0 to 3 meters in height. In response to the reviewer’s other query, collections were made mainly during the period of greatest abundance of migratory birds, and the time of sampling and sampling effort were the same in all localities.

Lines 260-262. This sentence needs to be rephrased. You have not demonstrated that migratory birds may disperse parasites (“demonstrate” followed by “may” is also confusing). I agree that you have shown that “lineages infecting both migrants and residents are more widespread than lineages restricted to residents”. However I don’t think you can separate whether this a result of migrants spreading these parasites, or that some lineages are just more common and thus more likely to infect both migrants and residents.

We understand the reviewer’s concern and have now replaced ‘demonstrate’ by ‘indicate’. We agree that our analyses we cannot definitely tell whether those parasites are being transmitted from the migrants to the resident community. Thus, we have rephrased this sentence L265. However, it is important to note that in our analyses we controlled for both parasite abundance (number of times each lineage was present) and generalism (number of avian species in which a lineage was found). Further, when considering the geographical range of lineages shared by resident and migrant species we excluded all the localities where the lineage was not found in the resident community (i.e. all localities where it was observed only in migrant hosts). Therefore, we expect the higher spread shown by the lineages shared by migrant and resident is most likely explained bydispersal through migrants.

One major finding discussed on lines 265 and 303-307 is the different effects of migrants on the prevalence of Plasmodium and Haemoproteus. This is interesting but to be confident that this is a solid biological finding, it would be good to know that this is not driven by the samples of the host species at the localities. Some species groups have more Haemoproteus others have more Plasmodium. Given the large number of host species sampled there must be quite different species compositions across the localities. If migrants are more common where the local birds have more Haemoproteus, it is of course an interesting association but the increased prevalence may not be due to the effect of the migrants.

We appreciate this comment and again understand the concern. Indeed, in our analyses we confirmed the existence of a considerable phylogenetic signal (0.49) and, therefore, we controlled our models for host phylogeny. In other words, even if the bird species involved in each of the parasite genus-specific analyses were a little different, we did our best to eliminate host phylogenetic effects from our assessment of the role of migration. Thus, we do not believe the difference in host composition is an issue in our results interpretation.

Lines 315-316. This is speculation that goes beyond your data – delete.

Deleted as suggested.

Line 317. Replace “demonstrate” with a less strong word. You just have a statistical association.

Modified as suggested. We replaced the word “demonstrate” with “suggest”

Line 353. Replace “demonstrate” for reasons given above.

Modified as suggested. We replaced the word “demonstrate” with “indicate”

Line 357. “resident host species harbour the greatest parasite richness…”. But the resident species makes up 90% of the species so not that surprising, unless you mean that this is per species.

We agree with the reviewer and have now deleted this sentence.

Line 362 “migrants appear to select bird communities”. Delete, you have no data to reach this conclusion

We understand the reviewer’s concern and have now rephrased this sentence to make it clear that this was only a suggestion and not an actual finding of this study.

Lines 364-367. This is not a suitable closing sentence of the study. It is well established in the literature that migrants can carry haemosporidian parasites throughout their migration routes, and if I understand the study correctly, you have not directly addressed this question (would require data from a migrant at breeding quarters, stopover sites and non-breeding (winter) quarters.

We appreciate this and all comments above. The closing sentence was rephrased. L368

**REVIEWER #2:**

In present study authors aim to clarify whether migratory birds influence the spread of haemosporidian parasites in South America and whether areas often crossed by migrants contain higher prevalence and richness of haemosporidians. They combined more than 13000 samples dataset and data obtained from MalAvi database. The results demonstrate that migrant birds may contribute to dispersal of haemosporidian infections. At the same time, it seems that migrant birds are present in regions with lower prevalence of Plasmodium and migrant birds can decrease the richness of haemosporidians.

In my opinion, this study contains a very good example of how merge of parasite’s data belonging to different parasite genus and generalization can cause false conclusions. For instance, in the analysis of haemosporidian parasites authors showed no correlation between the occurrence of migrants and the number of infected hosts. But when analysis was made separately for Plasmodium and Haemoproteus parasites, it was found that there are negative and positive relationships between migrants and prevalence of infection.

The manuscript in general is concise and clear, the conclusions are justified by data.

We thank the reviewer for their positive comments.

There are several points that should be addressed for consideration as well.

Authors use expressions throughout the text as “local percent of migrants” (Line 239) or “local number of infections” (Lines 234, 479, 483) which should be edited to “percent of migrants in area?” and “the number of local infections?”.

Replaced as suggested.

Line 310. Correct to …due to the fact that haemosporidians…

Modified as suggested

Line 325. Does any of these references contain information about the birds as well?

Altizer et al. 2011 is a review article that also presents information about migratory birds.

Line 358. Correct to …despite the fact that migrants…

Modified as suggested

Lines 481-482, 485-486, 487-488. Legends of figures should contain only explanatory information about the figure, but not the interpretation of the results.

Good point, this information has now been excluded as suggested

Figures 4 and 5 could be merged to one with A and B.

The figures have been combined as suggested.