**Responses to Reviewers’ Comments**

**Date**: November 7, 2023

**Manuscript Number**: AGEE38266

**Title of Article**: A predator in need is a predator indeed: generalist arthropod predators function as pest specialists at the late growth stage of rice

---------------------------------------------------------------------------------------------------------------------

Dear Dr. Audrey Alignier,

Thank you for inviting us to submit a revised version of the manuscript. We greatly appreciate the valuable comments and feedback from you and the reviewers. We have carefully considered each comment and incorporated most of the suggestions. In particular, we have made the following major changes:

* Included more details on the isotope sample preparation and MixSIAR model estimation.
* Provided the Bayesian posterior medians and 95% credible intervals of the dietary estimates from the MixSIAR model outputs as the supplementary material (Appendix B).
* Re-ran the beta regression models by including farm ID nested within pair ID as random effects and updated the methods and results accordingly.

Please also see the following section for our detailed point-by-point responses. All line numbers refer to the changes we made in the revised manuscript. We believe that the revisions based on the review comments have improved the quality of this manuscript, and we hope that the manuscript is now suitable for publication in *Agriculture, Ecosystems and Environment*.

---------------------------------------------------------------------------------------------------------------------

**Editor's comments:**

**Comment 1** > Thanks for your submission of a manuscript AGEE38266 titled ‘A predator in need is a predator indeed: generalist arthropod predators function as pest specialists at the late growth stage of rice’. The manuscript has been reviewed by two independent experts. As you will see from their comments copied below, reviewers find the topic of interest for AGEE readership. However, Reviewer#1 raised major concerns that I agree with especially about a better presentation of data on which tests were performed. Please consider presenting data in form of tables of medians and credible interval rather than means and standard errors; and for figures in form of posterior density plots rather than linear graphs. You must also better argue for the appropriateness of MixSIAR models. I also suggest to carefully edit the manuscript (l.219 : tillering ? and l.247 : tilling instead of tillering ?). Avoid to call for figures in the discussion section (main figures are more commonly associated with results). When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully. Outline every change made in response to their comments and provide suitable rebuttals for any comments not addressed.

**Response** > Thanks for the comments. The MixSIAR model estimation in this study was suitable because the three prey sources exhibited distinct signatures in the isotopic space (Appendix A: Fig. S1). Moreover, the model diagnostics showed that all mixing models have converged, further suggesting the appropriateness of these models.

The original outputs from the MixSIAR models were in the form of posterior means, posterior SDs, posterior medians, and 95% credible intervals, estimated for each combination of crop stage × individual farm. We extracted the posterior medians and calculated the means and SEs of the dietary proportions across the individual farms. Therefore, the means in the line graphs (Fig. 1 and 2) are not the posterior means *per se* but instead the means of the posterior medians (and the SEs are the SEs of the posterior medians). We feel that summarizing the original outputs across individual farms using line charts will better visualize the model results compared to having hundreds of posterior density plots for each combination of crop stage × individual farm. Nonetheless, to facilitate data transparency, we have now provided the posterior estimates from the original model outputs as the supplementary material (Appendix B).

Regarding the terminology, “tillering” would be an appropriate term in the context of our study. “Tilling” is a mechanical process that brings soil from below the ground to the surface and breaks/loosens it. This occurs at the beginning of the growing season before the seedling stage. On the other hand, “tillering” refers to the process in which rice plants start to branch from of the primary shoot following the seedling stage.

Finally, as suggested, we have removed all the references to main tables and figures in the discussion section (Appendix tables and figures were retained to provide necessary information).

**Reviewer's responses to questions**

1. Are the objectives and the rationale of the study clearly stated?  
  
Please provide suggestions to the author(s) on how to improve the clarity of the objectives and rationale of the study. Please number each suggestion so that author(s) can more easily respond.

Reviewer #1: Yes. More clear now.

Reviewer #2: Yes.

2. If applicable, is the application/theory/method/study reported in sufficient detail to allow for its replicability and/or reproducibility? Please provide suggestions to the author(s) on how to improve the replicability/reproducibility of their study. Please number each suggestion so that the author(s) can more easily respond.

Reviewer #1: Mark as appropriate with an X:  
Yes [] No [X] N/A []  
Provide further comments here:

**Comment 1** > The Bayesian mixing model MixSIAR works primarily within the framework of comparing relative importance of various food source options in consumers’ diets. While the model would work well for the herbivore food sources in predator diets (since we have multiple herbivores in the study), it is hard to see how it worked for herbivores as consumers given that only rice was considered as a source for herbivores.

**Response** > Yes, if we would like to quantify herbivores’ diet compositions, then plant sources other than rice should be included. Yet, here we focused only on predators’ diet compositions and thus this should not be a concern in our study.

**Comment 2** > The authors also need to state the sources of the means and standard deviation values of the various food sources (usually mandatory) that they applied to the MixSIAR models, and why they think these were appropriate for their particular study context.

**Response** > We have added a stable isotope biplot (mean and 95% CI) of the rice plant and the three prey sources in this study in our previous revision (Appendix A: Fig. S1). The isotopic signatures of the three prey sources were distinct enough, justifying the use of stable isotope mixing models.

**Comment 3** > In disputing the suggestion that a 1-km buffer is too large for most low-mobility arthropods with regards to trophic linkages, the authors refer to Rusch 2016 and Karp 2018 to maintain their stance. However, the two references relate to landscape-scale rather that plot-level or farm-level contexts which is the case for the present study. Also the full reference for Karp is not included in the bibliography.

**Response** > Thanks for pointing this out. We have now added Karp et al. (2018) to our reference. In fact, Rusch et al. (2016) stated in the methods section that 1-km spatial extent is an appropriate scale for examining enemy-pest trophic interactions, and they cited two other studies to support this argument:

*“The 1 km spatial extent was selected because it has been identified as a relevant scale to understand [trophic interactions](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/trophic-interaction" \o "Learn more about trophic interactions from ScienceDirect's AI-generated Topic Pages) and population dynamics for a range of organisms including natural enemies of [crop pests](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/crop-pest" \o "Learn more about crop pests from ScienceDirect's AI-generated Topic Pages) ([Thies and Tscharntke, 1999](https://www.sciencedirect.com/science/article/pii/S0167880916300512" \l "bib0200), [Thies et al., 2005](https://www.sciencedirect.com/science/article/pii/S0167880916300512" \l "bib0205)).”*

Even though our study focused on plot- and farm-level enemy-pest trophic interactions, the landscape-level pest control patterns in Rusch et al. (2016) and Karp et al. (2018) ultimately results from local trophic interactions, and therefore we feel that it is reasonable to examine whether there is any landscape-level effect on pest consumption by predators.

References:

Rusch, A., Chaplin-Kramer, R., Gardiner, M. M., Hawro, V., Holland, J., Landis, D., ... & Bommarco, R. (2016). Agricultural landscape simplification reduces natural pest control: A quantitative synthesis. Agriculture, Ecosystems & Environment, 221, 198-204.

Karp, D. S., Chaplin-Kramer, R., Meehan, T. D., Martin, E. A., DeClerck, F., Grab, H., ... & Wickens, J. B. (2018). Crop pests and predators exhibit inconsistent responses to surrounding landscape composition. Proceedings of the National Academy of Sciences, 115(33), E7863-E7870.

**Comment 4** > Like Reviewer#1 pointed out, and so did I in my initial review, the role of years on observed trends seems irrelevant, given that, firstly, agronomic practices on rice-fields of Taiwan are the same always and, secondly, years are not ecological variables such as habitat characteristics. The influence of years thus needs to be omitted completely, and instead use samples per year as replicates on the temporal scale. Implying observed trends across years to be indicators of potential use for climate tracking (as laid out in the Highlights) is too far-fetched. As the presented data from the GLM test show, year was not important as a factor anyway.

**Response** > We agree that year is not an ecological variable *per se*, but as mentioned in the discussion section of our manuscript, many biotic and abiotic factors can change from year to year, and we were interested in knowing whether pest consumption patterns varied or remained similar across the three study years (which did not). That said, we acknowledge that three years of climate data cannot be conclusive, and we have now modified the highlights, abstract, and conclusions to downplay our original statement.

**Comment 5** > In response to the query regarding use of spiders and lady beetles to generalize for all generalist predators, the authors argue that these two are considered common and thus representative of all generalist predators. This is not only wrong, given the diversity in foraging patterns amongst farmland predator groups, but also is compounded in this study by the various dimensions of explanatory factors: different farming systems; across multiple year; crop stages; vegetation structure; temperature etc. A further potential confounding element is, as reviewer#1 pointed out, the phenomenon of intra-guild predation, which many spiders are known for. In any case if the criterion was commonness, than ants are far more ubiquitous in all farming habitats than spiders and beetles, both on spatial as well as temporal scales. Why were ants left out?

**Response** > Thanks for the comments. Spiders and ladybeetles were indeed the most common generalist predators in our field samples, and they did consume high proportions of pest herbivores in their diets, thus serving as *promising* biocontrol agents in rice farms. Yet, we did not intend to argue that spiders and ladybeetles are *the only* biocontrol agents in the rice agro-ecosystems. Nonetheless, we have revised the manuscript to avoid over-generalizing our results.

As the reviewer mentioned, ants are common in agro-ecosystems and may also provide important regulation on pests. This is not mutually exclusive of our conclusion; ants, spiders, and ladybeetles can all serve as important biocontrol agents. However, our sweep net sampling method was not designed for surveying ants, and we did not collect many ants in the field samples. We acknowledge this limitation and have now added it to the potential caveats of this study (Line XXX). But even if we had collected abundant ants, their omnivorous nature would have prevented us from applying stable isotope mixing models to reliably quantify their diets. Molecular gut content analysis may be an alternative method, but this is outside of the scope of this study.

Regarding the issue of intra-guild predation, as addressed in the previous response, we were not able to estimate it in this study and we acknowledged this limitation in our previous revision (Line XXX).

**Comment 6** > In categorizing detritivores, the authors list some grasshopper groups, but leave out crickets. This is a bit worrying, given that crickets are among the most common detritivores (they are also omnivores) in rice farming systems. It is not reasonable for the authors to argue that crickets were excluded because they were rare in the farms studied.

**Response** > We believe that crickets are indeed more abundant in the farms. However, the sweep net sampling method was not designed for surveying ground-dwelling arthropods, and therefore we did not have many crickets in our field samples (which also prevented us from preparing sufficient stable isotope samples for them). We acknowledge this limitation and have now added it to the potential caveats of this study (Line XXX).

**Comment 7** > Also stating (retroactively) that only Hemiptera that consumed plant material were combined with grasshoppers for stable isotope analyses, without specifying which these were (after earlier stating that such combinations was done indiscriminately) only serves to raise suspicion about the data and analytical soundness for this study.

**Response** > We did not combine Hemiptera species and grasshoppers in our stable isotope analysis: Hemiptera species were categorized as “Rive herbivores”, whereas “grasshoppers” were categorized as “Tourist herbivores”. See *2.3. Arthropod trophic guild assignment* for more details.

Reviewer #2: Mark as appropriate with an X:  
Yes [] No [x] N/A []  
Provide further comments here:

**Comment 1** > L. 156. It is unclear how were the samples for the isotopic analyses made. Were all collected arthropods included in the samples or it was representative sub-samples that mirrored the composition proportionally?

**Response** > Thanks for pointing this out. Yes, the isotope samples were sub-samples of the field samples: We prepared isotope capsules for each species based on its relative abundance. For each field sample (arthropods collected at a crop stage in a single farm), we prepared up to 11 capsules for the most abundant species and 1 capsule for the species that were able to meet the minimum weight requirement for stable isotope analysis. We have now clarified the selection of arthropods for stable isotope analysis in the methods section (Line XXX).

3. If applicable, are statistical analyses, controls, sampling mechanism, and statistical reporting (e.g., P-values, CIs, effect sizes) appropriate and well described? Please clearly indicate if the manuscript requires additional peer review by a statistician. Kindly provide suggestions to the author(s) on how to improve the statistical analyses, controls, sampling mechanism, or statistical reporting. Please number each suggestion so that the author(s) can more easily respond.

Reviewer #1: Mark as appropriate with an X:  
Yes [] No [] N/A []  
Provide further comments here:

**Comment 1** > Good that beta regression with logit link function is now included in the analysis (Table 1). This is ok for the present purposes, even though beta regression would usually be most elegant for binary datasets. It is ok for here due to the use of logit (rather than log) link. Still, it is curious why, according to results of GLM analyses, Year was an important variable driving lady beetle predation rate.

**Response** > Thanks for the comments. A significant year effects on pest consumption by ladybeetle in the GLM results may be partially caused by the deviation in 2017 (Fig. 2), in which there were fewer replicate farms and ladybeetle samples for analysis.

**Comment 2** > If the authors have significant challenges handling MixSIAR modeling tools, it may be reasonable for them to call in technical support, or consider analyzing their data using alternative tools such as those involving molecular markers, specifically DNA meta-barcoding. However, while this will provide a thoroughly detailed resolution of trophic linkages (including at specie level), it will not estimate relative importance of food items in consumer diets the way MixSIAR can. Furthermore, DNA meta-barcoding would involve re-testing all the samples in the laboratory, with the obvious cost and time implications.

**Response** > We did not encounter significant challenges in running stable isotope mixing models. In fact, all of our models converged, indicating the appropriateness of these models. Molecular technique may be an alternative approach to examining trophic interactions and diet compositions, and it provides different information (e.g., detection rates of prey items) than stable isotope analysis does. This is not the main purpose of our study, but it may represent a future direction to better elucidate the pest consumption by GAPs in agro-ecosystems.

**Comment 3** > Like Reviewer#1, I have concerns as to why conclusions are drawn on observations from 4 crop stages yet results are presented inconsistently for 3 or 2 crop stages only growth stages. Was there anything wrong with data sets?

**Response** > We collected arthropod samples at four crop stages over the rice growth season and prepared isotope samples for all crop stages. However, there were not enough data points at the seedling stage for stable isotope mixing model estimation, therefore we excluded this stage in the subsequent diet composition and beta regression analyses (Line XXX). This is why there were only three crop stages (tillering, flowering, and ripening) presented in Table 3 (Tukey’s post-hoc tests for pest consumption among crop stages), Fig. 1 (diet composition by predators over the crop season), and Fig. 2 (pest consumption by predators over the crop season), but four crop stages (seedling, tillering, flowering, and ripening) in Fig. 3 (relative abundance of prey sources over the crop season).

**Comment 4** > With reference to Table 2 and Table 3: Tukey post-hoc tests are supposed to be POST-HOC tests of some original analytical procedures to resolve which factors has the greater weights than some other(s) one(s) in determining results obtained in ANOVA etc. Where are these original test results? Besides, Tukey post-hoc test results should return test statistics, p-values and degrees of freedom. Where are these in Table 2 and Table 3 results here?

**Response** > The original beta GLM model results were shown in Table 1. Because farm type and crop stage were significant (for Both predators), we proceeded to Tukey’s post-hoc comparisons of the predictor levels using the R package “emmeans”. We used a critical value approach with α = 0.05 for the post-hoc tests, and therefore no degrees of freedom and p-values were returned. Instead, the level differences were evaluated based on confidence intervals adjusted for multiple comparisons (as shown in Table 2 and 3).

Reviewer #2: Mark as appropriate with an X:  
Yes [] No [x] N/A []  
Provide further comments here:

**Comment 1** > The authors state that the design was paired (L. 132-136), nevertheless they did not account for the paired design in their statistical analyses as GLMs were used (L. 199). Instead GLMMs should be used with the pair ID as the random effect. Moreover, for me it is unclear whether the same fields or different fields were sampled across the three years. If the same fields were used, then the random effects should be field id nested within pair id.

**Response** > Thanks for the suggestions. We re-ran the beta regression models with individual farm nested within pair as random effects. The results are generally the same as previous ones. We have updated the methods and results accordingly.

4. Could the manuscript benefit from additional tables or figures, or from improving or removing (some of the) existing ones? Please provide specific suggestions for improvements, removals, or additions of figures or tables. Please number each suggestion so that author(s) can more easily respond.

Reviewer #1:

**Comment 1** > The current tables and figures present results of means and standard errors and line graphs yet for food proportions, these should be changed to median and credible intervals for tables, and probability density plots for figures. At least 1 table with isotopic signature values for consumers and food sources is also necessary at least as a supplementary material.

**Response** > The original outputs of the dietary proportions from the MixSIAR models were in the form of posterior means, posterior SDs, posterior medians, and 95% credible intervals, estimated for each combination of crop stage × individual farm. We then extracted the posterior medians and calculated the means and SEs of the dietary proportions across the individual farms. Therefore, the means in the line graphs (Fig. 1 and 2) are not the posterior means *per se* but instead the means of the posterior medians (and the SEs are the SEs of the posterior medians). We feel that summarizing the original outputs across individual farms using line charts will better visualize the model results compared to having hundreds of posterior density plots for each combination of crop stage × individual farm in the three study years. Nonetheless, to facilitate data transparency, we have now provided the posterior estimates from the original model outputs as the supplementary material (Appendix B) (Line XXX).

Reviewer #2: No.

5. If applicable, are the interpretation of results and study conclusions supported by the data? Please provide suggestions (if needed) to the author(s) on how to improve, tone down, or expand the study interpretations/conclusions. Please number each suggestion so that the author(s) can more easily respond.

Reviewer #1: Mark as appropriate with an X:  
Yes [] No [] N/A []  
Provide further comments here:

**Comment 1** > Spiders and ladybirds should not be construed to represent all generalist predators even if they are very common in rice-fields. Ants are more common than all the rest, yet they are not included here.

**Response** > We agree with the reviewer and have now revised the manuscript to avoid over-generalizing our results.

**Comment 2** > Observed yearly trends in the results should not be taken to imply corresponding climatic trends.

**Response** > We agree that three years of climate data may not be conclusive and we have now revised the manuscript to avoid over-inferring the relationship between climatic conditions and pest consumption patterns.

Reviewer #2: Mark as appropriate with an X:  
Yes [x] No [] N/A []

6. Have the authors clearly emphasized the strengths of their study/theory/methods/argument? Please provide suggestions to the author(s) on how to better emphasize the strengths of their study. Please number each suggestion so that the author(s) can more easily respond.

Reviewer #1:

**Comment 1** > The main strength of the study is in the use of stable isotopes to quantify trophic linkages in arthropods of rice-fields, which is novel, and in the characterization of the role of crop stages. But the authors seem to emphasize only the predation specialization aspect, which was not strongly supported by the study design and analytical procedures.

**Response** > Thanks for the comments. We did highlight the strength and novelty of this study regarding the use of stable isotopes to quantify trophic interactions in rice agro-ecosystems (Line XXX).

Reviewer #2: Yes.

7. Have the authors clearly stated the limitations of their study/theory/methods/argument? Please list the limitations that the author(s) need to add or emphasize. Please number each limitation so that author(s) can more easily respond.

Reviewer #1: Yes.

Reviewer #2: Yes.

8. Does the manuscript structure, flow or writing need improving (e.g., the addition of subheadings, shortening of text, reorganization of sections, or moving details from one section to another)? Please provide suggestions to the author(s) on how to improve the manuscript structure and flow. Please number each suggestion so that author(s) can more easily respond.

Reviewer #1: Generally yes.

Reviewer #2: No.

9. Could the manuscript benefit from language editing?

Reviewer #1: No.

Reviewer #2: No.

**Reviewer 1's comments:**

*Title*

**Comment 1** > The authors have made no attempt to modify any part of the title, in light of changes earlier suggested, and in light of their own admission that they focused on two predator groups rather than all generalist predators. Suggested title: "Spiders and lady-beetles consume higher proportions of rice pests at late growth stages regardless of farming system".

**Response** > Since the reviewer has a rather strong opinion on the title, maybe we can compromise a bit and modify the title as “A predator in need is a predator indeed: generalist arthropod predators (spiders and ladybeetles) specialize in consuming pests at the late growth stage of rice”.

*The Highlights*

**Comment 2** > The authors have made no changes to the here, in light of changes earlier suggested for study design, data analyses and results or conclusion. Observations across the three years cannot reasonably be used to imply the role of climate change in Taiwan.

**Response** > We agree that three years of climate data may not be conclusive and we have now revised the highlights accordingly.

*Abstract*

**Comment 3** > The authors have made absolutely no changes to the abstract, in light of changes earlier suggested for study design, data analyses and results or conclusion.

**Response** > We have now revised the abstract based on the reviewers’ comments and suggestions.

**Reviewer 2's comments:**

**Comment 1** > L. 207. Why only forest habitats and not also other habitat types?

**Response** > The landscape types surrounding our study farms are mostly farmlands and forested areas, with some human structures (houses and roads) scattering around. Since the landscape composition is relatively simple, we feel that quantifying the forest habitats will be sufficient to characterize the natural habitats around the study farms.

**Comment 2** > L. 211. Spiders can use the sit-and-move and active hunting too. For example, oxyopids and clubionids that were included in the analyses (Table S1) do not use sit-and-wait. Oxyopids use sit-and-move strategy while clubionids use active hunting. Just use different justification.

**Response** > Thanks for pointing this out. Yes, oxyopids and clubionids are not sit-and-wait predators. However, they only constituted a minor proportion of the spiders in the field samples (< 6%), and therefore we did not analyze their stable isotope signatures (and thus not included in all further analyses). We have now updated Table S1 (which shows all arthropods collected in the sweep net samples) to make this part clear.

**Comment 3** > L. 270. Paddy fields instead of agro-ecosystems.

**Response** > We have changed “agro-ecosystems” to “rice agro-ecosystems”.

**Comment 4** > L. 283. Forest cover instead of surrounding landscape.

**Response** > We have changed “surrounding landscape” to “forest cover”.

**Comment 5** > L. 380-381. Actually spiders are very effective in suppressing pests in rice fields in comparison to other crops and this study shed some light on why. See the meta-analysis Michalko et al. 2019, Glob. Ecol. Biogeogr. 28(9): 1366-1378.

**Response** > Thanks for the positive comments.