**Response to Reviewers’ Comments**

**Reference number**: JAPPL-2024-00547

**Title of article:** Generalist predators function as pest specialists: examining diet composition of spiders and ladybeetles across rice crop stages

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Dear Dr. Cate Macinnis-Ng,

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 suggestions. In particular, we have made the following major changes:

* Clarify the sample size issues and revised the replication statement
* Addressed the issue of IGP and discuss it in more details
* Perform a sensitivity analysis of TDFs
* Strengthen the theory regarding predator-prey interactions and pest control in the introduction

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 greatly improved the quality of this manuscript, and we hope that the manuscript is now suitable for publication in *Journal of Applied Ecology*.

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**Editor's comments**

**Comment 1** > Following the evaluation of the manuscript titled "Generalist predators function as pest specialists: examining diet composition of spiders and ladybeetles across rice crop stages" by the three reviewers, we have concluded that a major revision is necessary before we can consider the publication of this study.

The first reviewer acknowledged the interesting narrative regarding the diet composition shift of generalist arthropod predators over the rice growth period and their potential utility in biocontrol. However, several concerns were raised, including insufficient sample size, which compromises the robustness of the conclusions, and the similarity in methodology and results with a previously published study, raising questions about the originality of the work. The reviewer also suggested minor clarifications and details needed in various sections of the text.

The second reviewer appreciated the impressive dataset collected and the relevance of the research to the natural pest control literature but pointed out a lack of ecological theory and deficiencies in the data analyses that affect the interpretation of the results. Additionally, there were critiques regarding the clarity of the figures and tables presented.

The third reviewer praised the clarity and conciseness of the writing and the relevance of the topic. However, significant issues were noted in the study design and statistical methodologies. Specifically, the reviewer highlighted the absence of non-herbivore resources in the Bayesian mixing model analysis, which can affect the accuracy of results due to intraguild predation and cannibalism, especially in spiders. Moreover, the methodology used for calculating trophic discrimination factors (TDFs) is contested in the literature and needs to be reviewed.

Given the collective feedback, it is evident that while the study has substantial merits and contributes to understanding the role of generalist predators in biological control, significant revisions are necessary to address the raised concerns. I strongly recommend that the authors:

- Reassess and, if possible, increase the sample size per site to strengthen the conclusions.

- Include other potential non-herbivore prey in the Bayesian mixing model analysis, considering the complex diet of predators like spiders.

- Review the methodology for calculating TDFs, considering existing criticisms, and, if necessary, use pre-established TDF values for the studied taxa from the literature.

- Address the issue of intraguild predation and its implications for biological control more thoroughly in the discussion.

- Respond to specific minor critiques from the reviewers regarding clarity and detail in the manuscript.

I hope that these modifications can be implemented to improve the quality and robustness of the manuscript, ensuring it makes a significant and reliable contribution to the scientific literature on biological control.

**Response 1** >

Thanks for the positive and constructive feedback from the editor and the reviewers. We have provided our point-by-point responses to all the comments and concerns raised by the reviewers in the following section. Regarding the key recommendations from the editor, we summarize the major changes made in this revised manuscript:

- Reassess and, if possible, increase the sample size per site to strengthen the conclusions.

> We performed stable isotope mixing model analysis separately for spiders, ladybeetles, and both predators combined, and the number of sites × crop stages over the three study years were 86, 57, and 94 for “Spiders”, “Ladybeetles”, and “Both predators”, respectively (the sum of the last column in Table 2), not 237 as pointed out by Reviewer 1. Among the 352 predator isotope capsule samples, 252 were spiders and 100 were ladybeetles, and therefore there were on average 252/86 = 2.9 predator data points per site × stage × year in the model for “Spiders”, 100/57 = 1.8 predator data points for “Ladybeetles”, and 352/94 = 3.7 predator data points for “Both predators”.

Each predator isotope capsule may contain one or multiple predator individuals (depending on their dry weight), so the actual numbers of predator individuals involved in the stable isotope analysis were higher than the numbers shown above, and the isotope signatures of predators in the mixing models indeed represented a wider range of individuals in the population. Moreover, the MCMCs in our Bayesian stable isotope mixing models all converged, indicating that the data inputs were adequate and the model results were reliable. Therefore, we feel that our interpretation of the results and the conclusions made accordingly are appropriate.

As suggested by Reviewer 1, we have added the number of isotope capsules for the predators and prey sources to Table S1.

- Include other potential non-herbivore prey in the Bayesian mixing model analysis, considering the complex diet of predators like spiders.

> In fact, we included three prey guilds in our mixing model analysis for both spiders and ladybeetles: rice herbivores, tourist herbivores, and detritivores, which are non-herbivore prey

> Clarify the prey guilds in the methods

- Review the methodology for calculating TDFs, considering existing criticisms, and, if necessary, use pre-established TDF values for the studied taxa from the literature.

> Read Caut et al. and provide justifications for using DDDFs for the mixing model instead of the pre-established TDFs (cite papers that criticize the use of pre-established TDFs)

> Add the details in the methods section and provide a summary table of TDFs used in the supplementary information

> There is no pre-establish TDFs for some of the prey taxa in our analysis

- Address the issue of intraguild predation and its implications for biological control more thoroughly in the discussion.

> Make IGP a standalone paragraph in the discussion and talk about its significance and the limitations of our study

- Respond to specific minor critiques from the reviewers regarding clarity and detail in the manuscript.

> We have provided our detailed point-by-point responses to all the comments and concerns raised by the reviewers in the following section.

**Reviewer 1's comments**

**Comment 1** >

The study titled "Generalist predators function as pest specialists: examining diet composition of spiders and ladybeetles across rice crop stages" shows an interesting story of how the diet composition of generalist arthropod predators switches more and more to rice herbivores during the rice growth period, suggesting their usefulness in biocontrol during the most critical crop stages when the densities of rice herbivores are high. The study also investigated the effect of different abiotic and biotic factors that could affect the diet composition, suggesting that predators in conventional farms prey more on rice herbivores and that the predation trend through crop stages is constant through the years. This suggests that spiders and ladybugs are consistent in their predation behavior inside rice fields, which could lead to the development of stable biocontrol practices.

The manuscript is well-written, with a well-elaborated and interesting introduction. The Discussion could focus slightly more on comparing the results of their study with those of different studies, but otherwise, they explained what needed to be explained. I added minor comments on several sentences that I believe should be addressed and clarified.

**Response 1** > Thanks for the positive feedback and we have addressed the comments below and revised the manuscript accordingly.

**Comment 2** >

Sample size. If I understood correctly, based on Table S2, you had 240 replicates of sites over all crop stages and years (I summed up the Ns in Table S2). And you stated that you processed 352 predators for the analysis. This means that per each site, you had, on average, 1.46 specimens. I guess that the number is larger than 1 because for some samples in the site, you added two individuals because one was not enough in mass, but this means that you had only one specimen per site for the analysis, which also explains why in some charts, especially those for ladybugs, I do not see any error bars for the relative proportion of prey sources in the diet. With that said, we are assuming the diet composition here is based on just one or a few individuals and that they represent the whole site. Even if the data is consistent through 3 years, there is still insufficient evidence to support the conclusions. The sample size should be much higher per site to draw such conclusions. If the sample size is larger and I misunderstood the methodology section, please write in Table S1 the number of individuals of each predator you investigated.

**Response 2** >

The stable isotope mixing model analysis for the dietary proportions were done separately for both predators combined, spiders only, and ladybeetles only, and the number of sites × crop stages over the three study years were 94, 86, and 57 for “Both predators”, “Spiders”, and “Ladybeetles”, respectively (sum of the last column in Table 2), not 237. Among the 352 predator isotope capsule samples, 252 were spiders and 100 were ladybeetles, and therefore there were on average 352/94 = 3.7 predator data points in the mixing model per site × stage × year for “Both predators” (not 1.46 as calculated), 252/86 = 2.9 predator data points in the model for “Spiders”, and 100/57 = 1.8 predator data points in the model for “Ladybeetles”.

In fact, our field arthropod samples actually contained more predator individuals than the number of isotope capsules. However, because of the budget limitations and time constraints, we were not able to prepare isotope capsule samples for all predator individuals sampled. That said, each predator isotope capsule may contain one or multiple predator individuals (depending on their body mass), so the actual numbers of predator individuals involved in the stable isotope analysis were indeed higher than the numbers of predator capsules shown above. Consequently, the isotope signatures of predators in the mixing models represented a wider range of individuals in the field.

As suggested, we have now added the number of isotope capsules for the predators and prey sources to Table S1.

**Comment 3** >

2) This study has a similar methodology, results and conclusions as the one published in 2021 (https://doi.org/10.1002/ecs2.3625), which you cited as a data source. The larger difference in the methodology that I could recognize between this manuscript and the published study is that you collected over three years instead of one year. However, the middle year is the same in both studies. I am curious if you used the same dataset for the year 2018 in both studies or if you used other samples. If that is the case, it would be good to mention it.

**Response 3** > Yes, the samples in year 2018 in this study were the same as those in the previous study by Hsu et al. (2021). We have now mentioned this in the methods section (line XXX).

*Minor comments*

**Comment 4** > Line 8: term "recent Anthropocene" could mean quite a large period, depending on who you ask. Rephrase it to the "recent decades".

**Response 4** > We have revised this.

**Comment 5** > Line 17: As you decided not to focus on the seedling crop stage due to insufficient data (I am just not sure which data was insufficient, which could be mentioned in more detail), you can remove it from here.

**Response 5** > Thanks for the suggestions. In fact, we prepared stable isotope samples for arthropods collected at the seeding stage, but the isotope data points were not enough for reliable mixing model estimation of predators’ diet composition (missing prey sources and low predator number). We have added some details to the methods section to explain this situation (line XXX). Although we did not analyze the predators’ diet composition, we still provided the arthropod abundance data at the seeding stage (figure SX), and therefore we feel that it is appropriate to mention seeding stage in the abstract (to avoid potential confusions).

**Comment 6** > Line 51-53: Missing citation for this sentence.

**Response 6** > We have added the citations for it (line XXX).

**Comment 7** > Line 111 - 113. This assumption (or hypothesis) was not elaborated here as to why you expected it.

**Response 7** > We have revised this statement (line XXX).

**Comment 8** > Line 257: I am missing any table with the results of the forest cover for each of the sites or pair of sites.

**Response 8** > We have provided the percent forest cover for each study farm in the supplementary Table SX.

**Comment 9** > Line 330: "... for pest control in rice fields".

**Response 9** > We have revised this.

**Reviewer 2's comments**

**Comment 1** >

This study presents diet analyses of spiders and ladybeetles over three years across organic and conventional rice farms, particularly investigating the proportion of pests in their diet over the season and across years. The authors use isotopes to assess diet. I am not an expert in these methods. The paper has collected an impressive dataset on predator diets, which is dearly missing from the natural pest control literature. I think it is very valuable work with a solid sampling design. However, the paper lacks ecological theory, and I have some concerns regarding the data analyses and thus interpretation of the results that need to be addressed before accepting this manuscript.

**Response 1** > Thanks for the positive feedback and we have addressed the comments below and revised the manuscript accordingly.

**Comment 2** > The introduction lacks **links to theory**, predator-prey cycles, and prey diversity-predation. The impact of seasonality (via changes in prey abundances, richness, intraguild predation), years, and of organic/conventional systems on diet composition needs to be better introduced and linked to current research. Currently, it lacks background, and the hypotheses are a bit weak.

**Response 2** >

> Add details to the introduction for how growing season, year, and farming practice affect diet compositions

**Comment 3** > The **isotope method** used to assess diet needs to be introduced before the end of the introduction and compared to other available methods. What can we extrapolate using isotopes, and to what taxonomic level? As I am not an expert, it was very difficult for me to understand what this method can and cannot distinguish in terms of diet (presence/absence data, at the species/family level?).

**Response 3** > Stable isotope analysis has been widely used in trophic ecology to estimate the diets of predators from known prey sources. Depending on the research questions, “predators” can be the individuals within a species, a family, or a trophic guild. For example, if one is interested in the overall diet composition of in the ladybeetle family, one can collect different individuals of different ladybeetle species, analyze their stable isotope signatures, and run stable isotope mixing models to estimate the diet composition of an average/typical individual in the ladybeetle family. By suppling the isotope signatures of the potential prey items the predators might consume, the mixing models can estimate the proportions of these prey items consumed in the predators’ diets.

Compared to other common techniques such as field observations of predators feeding behavior and molecular gut content analysis, which gives the absence/presence of prey items in the predators’ diets, stable isotope analysis can provide “quantitative” information on prey items consumed (the proportions of different prey items in the total diets of predators). Moreover, instead of the “snap-shot” information gleaned from field observations and molecular gut content analysis, stable isotope analysis provides time-integrated dietary information (i.e., the average dietary proportions from different prey items over a period of time, with the length of this period depending on the tissue assimilation and turnover rates), allowing for inferences about predator-prey trophic interactions from a different perspective.

We agree with the reviewer that it would be great to introduce the isotope method earlier so that the readers, especially those who are not familiar with it, can get a better idea of this approach. We have now clarified this part and brought it up immediately after we mention stable isotope analysis in our study (line XXX).

**Comment 4** > **Classification of predators:** why consider ladybeetles as generalist predators? They are considered by natural pest control experts as predator specialists (Riggi et al., Ecological Indicators 2024). This is also the reason for the greater pest presence in their diets compared to more generalist and opportunistic predators such as spiders. Additionally, spiders constitute a varied group with different hunting modes that likely feed differently. As individuals were identified to species or families, this should be investigated (Sanders et al. 2015, <https://besjournals.onlinelibrary.wiley.com/doi/pdf/10.1111/1365-2656.12271>). This could lead to a more ecological approach to pest control and uncover mechanisms affecting diet composition by looking into traits: Generalist versus specialist (spiders vs. ladybirds) , Hunting mode within spiders

**Response 4** >

> Check the references to see if ladybeetles are generalists or specialists? Find other references/review papers to support our study. Justify why we did not compare generalist vs. specialist

> The dietary patterns of ladybeetles are pretty diverse and can range from specialists to generalist and even omnivores (citations). The dominant species in our system, the orange ladybeetle, has been shown to be a generalist predators (citations), and we feel that it is appropriate to refer to them as generalists

> The spiders are a diverse group of generalist arthropod predators and different groups have different hunting modes and foraging patterns. In our system, the most dominant spider species is the long-jaw orb weaver, which constitutes over XXX% of the spider individuals in our samples, whereas other spider species (e.g, lynx spider) were relatively uncommon in rice farms. We therefore pooled all spiders into the same predator guild because we did not have enough samples for stable isotope analysis and reliable mixing model estimation for individual spider groups. Moreover, our goal is to understand the guild-level trophic interactions patterns, and therefore pooling may be appropriate for this purpose. However, we have now acknowledged this in the limitations. Future studies can implement more elaborate sampling design to focus on different spider groups and understanding the within-guild variation in diet composition

> Check the hunting mode of the spiders in our isotope samples

> Take the isotope values from the two spider groups, and see if they differ, If not, then it’s fine to pool them together.

**Comment 5** > **Analyses:** Whyprey and predator abundances in the field were not included in the model to see if the diet composition was representative of the absolute field abundances rather than the relative? Also to be able to interpret the results it would be relevant to check if prey abundances and predator abundances were affected by farming type\*landscape and year as well, not only the diets.

**Response 5** > Thanks for the suggestion. We refit the beta GLMMs using the absolute abundance instead of relative abundance of rice herbivores in the field as the predictor for “Both predators”, “Spiders”, and “Ladybeetles”. Overall, the model results were qualitatively similar and did not alter our original conclusions:

|  |  |  |
| --- | --- | --- |
|  | Relative abundance of rice herbivores | Absolute abundance of rice herbivores |
| Both predators | *β* (± SE) = –0.23 (± 0.35)  *χ*2 = 0.56, *P* = 0.46 | *β* (± SE) = –0.0006 (± 0.0006)  *χ*2 = 0.90, *P* = 0.34 |
| Spiders | *β* (± SE) = 0.31 (± 0.41)  *χ*2 = 0.58, *P* = 0.45 | *β* (± SE) = –0.00001 (± 0.0008)  *χ*2 = 0.0002, *P* = 0.99 |
| Ladybeetles | *β* (± SE) = 0.13 (± 0.22)  *χ*2 = 0.38, *P* = 0.54 | *β* (± SE) = 0.0003 (± 0.0005)  *χ*2 = 0.35, *P* = 0.55 |

Research has shown that the relative abundance of prey can predict diet variation and prey switching in generalist predators (Coblentz 2020), and a study on an acarine predator also suggests that the relative abundance of prey is more important than the absolute abundance in terms of prey choice and consumption (Walde et al. 1995). Therefore, we feel that using the relative abundance of rice herbivores as the predictor in the model is biologically meaningful in terms of predators’ foraging behavior and dietary patterns.

We also refit the models with the absolute predator abundance included as an additional predictor. The results suggest that predator abundance did not have a significant impact on pest consumption by predators. Since our main objective was to examine the effect of prey abundance (rather than predator abundance) on pest consumption by predators, we decided not to alter the model structure in our original analysis.

|  |  |
| --- | --- |
|  | Absolute predator abundance |
| Both predators | *β* (± SE) = –0.009 (± 0.006)  *χ*2 = 2.1, *P* = 0.14 |
| Spiders | *β* (± SE) = –0.001 (± 0.007)  *χ*2 = 0.03, *P* = 0.87 |
| Ladybeetles | *β* (± SE) = –0.006 (± 0.004)  *χ*2 = 2.7, *P* = 0.10 |

> We also analyze the effect of farm type and landscape on predator and prey abundance and updated the methods and results section

References:

Coblentz, K. E. (2020). Relative prey abundance and predator preference predict individual diet variation in prey‐switching experiments. *Ecology*, *101*(1), e02911.

Walde, S. J., Magagula, C. N., & Morton, M. L. (1995). Feeding preference of Zetzellia mali: does absolute or relative abundance of prey matter more?. *Experimental & applied acarology*, *19*, 307-317.

**Comment 6** > **Abstract:** Lacks clarity in the methods (seedling stage was not analyzed, how many fields, what is meant by biotic/abiotic factors, how were the arthropods collected) and results (what do the percentages correspond to? what does higher mean?) and the conclusion is a bit vague.

**Response 6** > Thanks for pointing these out. We have added the method details and clarified the results and conclusion. Please see the revised abstract.

*Specific Line Comments*

**Comment 7** > L. 66: I do not agree that we still need to validate “spiders and ladybird biocontrol potential” – However, this paper could bring a better understanding of how pest and prey abundances affect diet composition and stability. Same L.91

**Response 7** > Thanks for the suggestion and we have now revised the sentence accordingly (line XXX and line XXX).

**Comment 8** > L. 78: Consider replacing reliability with stability or variability.

**Response 8** > We have replaced reliability with stability (line XXX).

**Comment 9** > L. 85: What is meant by abiotic and biotic factors?

**Response 9** > We have revised this (line XXX).

**Comment 10** > L. 93: Can you define what you mean by diet/consumption composition?

**Response 10** > Diet composition in this study means the proportions of different prey items consumed in predators’ diet. We have now added this definition (line XXX).

**Comment 11** > L. 113: Unclear hypothesis. I would expect that predation by generalists would depend mostly on pest abundances rather than crop stages.

**Response 11** > We agree that the dietary patterns of generalist predators is largely governed by the prey abundance. However, farm factors such as farming practice and crop stage can also influence predators’ foraging behavior by altering the prey availability in the field. In particular, prey composition and pest abundance change substantially over the growing season as crops develop, and therefore we would expect pest consumption by predators to vary across crop stages.

**Comment 12** > L. 117: What do you mean by proportional contribution? Different prey sources? Define (is this per predator individual or per group).

**Response 12** > Stable isotope mixing models estimate the proportions of different prey items (sources) in predators’ diet in terms of biomass based on the isotope signatures of prey and predators (e.g., 30% of total biomass consumed by the predator comes from prey A, 50% from prey B, and 20% from prey C). In general, the isotope signatures of multiple predator individuals (from a species or a researcher-defined trophic guild) are used as the input for the mixing models, and therefore the model results represent the dietary patterns of predators at the population/guild level. We have now clarified this (line XXX).

**Comment 13** > Very nice design but missing the number of fields sampled in pairs.

**Response 13** > We did mention in the sub-section *2.1* S*tudy system and sample collection* that we had 3 farm pairs in 2017 and 7 farm pairs in 2018 and 2019 (line XXX).

**Comment 14** > Consider including a map.

**Response 14** > We have now included a map of the study farms in Fig. X.

**Comment 15** > Include information on landscape factors around the pairs (min/max/mean/sd).

**Response 15** > We have provided the percent forest cover of all 7 farm pairs in the supplementary Table SX.

**Comment 16** > Include a sampling design in the appendix and maybe figures of the different stages.

**Response 16** > We have now included the sampling design and photos of the major rice stages in this study in Fig. X.

**Comment 17** > Were the samples from each transect merged?

**Response 17** > Yes, the arthropod samples from the two transects during each farm survey were pooled (line XXX).

**Comment 18** > Organisms were counted and identified in each transect? This is amazing data, and I do not understand why in the analyses: Spiders were all blended into one single group? Use traits or family level to investigate diets in more detail.

**Response 18** > Yes, arthropod samples from the two transects were pooled and later identified to the family level (or to genus level if possible) and tallied. Also see our response to comment 17.

The spiders in our field arthropod samples consisted mainly of two families: Araneidae and Tetragnathidae. Because our objective was to understand the overall dietary composition and pest consumption patterns of spiders as a whole rather than individual spider groups, we pooled these two spider families as a single trophic group in our stable isotope mixing model analysis. However, we agree that future research on the diets of different spider groups will provide a fine picture of their interactions with pests and help better understand their pest control services in the farms. Please also see our response to comment 4 for more details.

**Comment 19** > Prey and predator abundances in the field were not included in the model to see if the diet composition was representative of the absolute field abundances rather than the relative?

**Response 19** > Please see our response to comment 5 for details.

**Comment 20** > Why were prey grouped? Could network analyses not be used? What is the taxonomic level that can be distinguished using isotope analyses? And what is the data coming out (is it like molecular gut content absence/presence)?

**Response 20** > Stable isotope mixing models are models that estimate the proportions of different resources in consumers’ diet. In general, the models can reliably estimate the dietary proportions for at most *n*+1 resources if there are *n* isotope elements. In our study, we have two isotopes (13C and 15N), allowing us to input three prey sources into the models as the resources for predators. However, because our field samples consisted of many families of prey (Table SX), we combined them into three trophic guilds (trophic species) and use these three prey guilds as the prey data input (also see the sub-section *2.3. Arthropod trophic guild assignment* for more details). In fact, the prey taxonomic levels used in the mixing models will depend on the study systems and research questions. If one is interested in the dietary proportions of predators from a few specific prey species (which is not the case for our study), then the isotope data of these individual prey species can be used in the models as the resources for predators.

The output of mixing models is the proportions of different prey sources (items) consumed in the total diet (based on biomass) of an average individual in the focal predator species or trophic group. For example, in a mixing model with isotope data on three prey sources A, B, and C as well as one predator species D, the model will yield estimates of the proportions of prey sources A, B, and C in the diet of the predator D, and these proportions represent the diet of an average or a “typical” individual in the population of the predator D.

**Comment 21** > L. 147: How common was it to add several conspecifics to one capsule? I wonder then what the percentage means, as I imagine smaller species will need to be more often combined than larger ones. Doesn’t that create a bias? Why not use a % per biomass rather than % per capsule? Unclear how this was dealt with.

**Response 21** > For stable isotope analysis, there is a minimum amount of dry tissue required to get reliable estimates of 13C and 15N depending on the material type and the C and N contents (https://stableisotopefacility.ucdavis.edu/sample-weight-calculator). In our study, except for a few large-bodied families such as Acrididae, we generally oven-dried several individuals, pulverized them, and weighed around 2 mg of dry tissue into each isotope capsule. The smaller-bodied families did require more individuals to meet the weight. However, we prepared multiple isotope capsules for each family if possible, and these “capsule-level” replicates could help reduce the potential bias due to unequal pooling of individuals in the capsules.

The isotope capsules samples were combusted in an analyzer to get the 13C and 15N contents. Because these values are extremely small in the absolute sense, they are expressed as isotopic deviations from the international standards (the delta δ values) with a unit of per mill (‰). Please see the website for more explanations on stable isotope analysis (https://www.nature.com/scitable/knowledge/library/the-use-of-stable-isotopes-in-the-96648168/).

**Comment 22** > L. 156: Please provide a table with the species in each category.

**Response 22** > We did provide the families/genera in each trophic guild in Table SX in the first submission.

**Comment 23** > L. 162: I am not clear how you can distinguish between the species (rice pests and other herbivores).

**Response 23** > The assignment of rice herbivores (pests) and other herbivores (tourist herbivores) was based on a combination of literature surveys and k-means clustering of their stable isotope signatures (line XXX). We first did a general classification by searching the literature for the diets of the herbivore families/genera, and then analyzed their stable isotope signatures to confirm our assignment. Rice herbivores and tourist herbivores can be distinguished in an isotope biplot (Figure SX) because they feed on plant sources with different isotope signatures (rice plant is C3 while the grasses in surrounding vegetation are C4).

**Comment 24** > L. 163: Instead of tourist herbivore, replace it with “alternative prey.”

**Response 24** > Thanks for the suggestion. We feel that using “tourist herbivore” in this study is appropriate for two reasons. (1) We had two alternative prey guilds (“tourist herbivore” and “detritivore”) and we would like to distinguish between them. (2) We would like to distinguish between herbivores that feed primarily on rice plant (rice herbivores) and those that feed primarily on other plant sources in the surrounding vegetation (tourist herbivores).

**Comment 25** > L. 178: What are “mixing models”?

**Response 25** > Stable isotope mixing models are models that estimate the diet of a consumer individual (or a group of consumer individuals) based on the isotope signatures of their tissues and food sources (Parnell et al. 2013). They should not be confused with mixed models, which are statistical models for assessing predictor effects on the response.

Reference:

Parnell, A. C., Phillips, D. L., Bearhop, S., Semmens, B. X., Ward, E. J., Moore, J. W., ... & Inger, R. (2013). Bayesian stable isotope mixing models. *Environmetrics*, *24*(6), 387-399.

**Comment 26** > L. 178: I am not sure I understand why Bayesian was needed. I am not familiar with these methods, and a little introduction on what these methods do and why using this would be nice. Also, why not include a random factor? Farm ID and farm pair?

**Response 26** > We used the R package MixSIAR for our mixing model analysis. The package implements a Bayesian approach to estimating the dietary proportions of predators. Bayesian framework has the advantage of incorporating prior information regarding the diets of predators and various sources of uncertainties in the diet estimation (Moore & Semmens 2008, Parnell et al. 2013). We have added a brief introduction of the Bayesian approach to the methods section (line XXX).

Regarding the random effects, we did include farm ID nested within farm pair ID as the random effects in our beta regression models (line XXX).

Reference:

Moore, J. W., & Semmens, B. X. (2008). Incorporating uncertainty and prior information into stable isotope mixing models. *Ecology letters*, *11*(5), 470-480.

Parnell, A. C., Phillips, D. L., Bearhop, S., Semmens, B. X., Ward, E. J., Moore, J. W., ... & Inger, R. (2013). Bayesian stable isotope mixing models. *Environmetrics*, *24*(6), 387-399.

**Comment 27** > L. 183: What is C and N dependencies? That sentence is not clear.

**Response 27** > C and N dependencies refer to the C and N contents of the isotope samples. Because the C and N contents vary among organisms, incorporating this source of variation in the mixing models can help improve the accuracy of the diet estimates. We have now clarified this in the methods section (line XXX).

**Comment 28** > L. 185: What is a trophic discrimination factor?

**Response 28** > A trophic discrimination factor (TDF ∆) is defined as the difference in isotopic values between the prey source and the consumer. This isotopic difference arises from fractionation, the preferential utilization of lighter over heavier isotopes of an element in organisms’ tissues, leading to increasing δ13C and δ15N values at higher trophic levels. Stable isotope mixing models require TDFs to adequately account for such changes in δ13C and δ15N when estimating the diet compositions of consumers.

**Comment 29** > L. 188: Add reference.

**Response 29** > We have added the reference (line XXX).

**Comment 30** > L. 189: Add reference.

**Response 30** > We have added the reference (line XXX).

**Comment 31** > L. 193: Why not include year as an interaction with farm type if you are interested in stability over time? Also, I am not sure why you use relative abundance rather than absolute? And why not include predator abundances?

**Response 31** > We appreciate the suggestions for model fitting. We did not include farm type × year interaction in the beta GLMMs because we were not interested in whether the “difference” in pest consumption by predators in organic and conventional farms (i.e., the effect of farm type) varied across the three study years. Instead, we were more interested in whether the “overall” pest consumption patterns in both organic and conventional farms varied across years, and therefore we included only the main effect of year in the models.

Regarding the relative and absolute abundance of rice herbivores, we refit the GLMMs using the absolute abundance instead of the relative abundance, and the results were qualitatively similar. Please see our response to comment 5 for more details.

Regarding the predator abundance, we refit the GLMMs with the absolute predator abundance (the number of predator individuals in the field samples) included as an additional predictor. The results suggest that predator abundance did not have a significant impact on pest consumption by predators. Please see our response to comment 5 for more details.

**Comment 32** > I would be interested to know if prey abundances and predator abundances were affected by farming type\*landscape and year as well, not only the diets.

**Response 32** > Thanks for the suggestions. We fit negative binomial GLMMs with the predator (both spiders and ladybeetles) and the rice herbivore abundance (the number of individuals in field samples) as the responses, farm type, crop stage, and year as the fix effects, and farm ID nested within farm pair ID as the random effects. The number of observations in each year was used as the weights in the models. The results show that predator abundance varied among years (2019 > 2017 and 2018) and crop stages (tillering > ripening > flowering) but did not differ between organic and conventional farms. Rice herbivore abundance also varied among years (higher in 2019 compared to 2017 and 2018) but did not vary among crop stages or differ between organic and conventional farms. Percent forest cover did not have a significant effect on both predator and rice herbivore abundance.

> Update the methods section and add the results to supplementary Table SX

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model | Factor | *d.f.* | *χ*2 | *P* |
| Predator abundance (both spiders and ladybeetles) | Year | 2 | 64.2 | < 0.001 |
|  | Farm type | 1 | 0.006 | 0.94 |
|  | Crop stage | 2 | 40.0 | < 0.001 |
|  | Percent forest cover | 1 | 1.8 | 0.18 |
| Rice herbivore abundance | Year | 2 | 30.2 | < 0.001 |
|  | Farm type | 1 | 0.63 | 0.43 |
|  | Crop stage | 2 | 1.0 | 0.60 |
|  | Percent forest cover | 1 | 2.8 | 0.10 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model | Farm type | EMMs (± SE) | Lower 2.5% | Upper 2.5% |
| Predator abundance | Organic | 7.6a (± 1.2) | 5.7 | 10.3 |
|  | Conventional | 7.7a (± 1.1) | 5.8 | 10.3 |
| Rice herbivore abundance | Organic | 41.2a (± 8.1) | 28.0 | 60.6 |
|  | Conventional | 50.0a (± 9.8) | 34.0 | 73.4 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model | Farm type | EMMs (± SE) | Lower 2.5% | Upper 2.5% |
| Predator abundance | Tillering | 13.8a (± 2.2) | 10.2 | 18.8 |
|  | Flowering | 4.4b (± 0.7) | 3.2 | 6.1 |
|  | Ripening | 7.4c (± 1.4) | 5.2 | 10.6 |
| Rice herbivore abundance | Tillering | 39.8a (± 8.0) | 26.8 | 59.0 |
|  | Flowering | 48.0a (± 8.9) | 33.4 | 69.0 |
|  | Ripening | 48.9a (± 10.5) | 32.1 | 74.6 |

**Comment 33** > L. 210: Replication statement should be N = XX number of paired fields, not N number of individuals analyzed, I think.

**Response 33** > Thanks for pointing this out and we have now revised our replication statement (line XXX). In fact, our analyses consisted of two parts. The first part was the stable isotope mixing models for quantifying the diet compositions of predators in rice farms, and the number of replicates was the number of predator and prey isotope capsules analyzed. The second part of the analyses was the pest consumption by predators in relation to various factors (farm type, crop stages, year, and relative abundance of rice herbivores), and the number of replicates at this scale would be the number of crop stage × study farm × year.

**Comment 34** > The results section is very descriptive, with no analyses results present and measures of variation lacking.

**Response 34** > In the first two sub-sections of the results, we presented the dietary proportions of predators obtained from the mixing models, which were more descriptive in nature. We provided the uncertainties around these estimates in the supplementary Table SX. In the third sub-section of the results, we presented the effects of various factors on pest consumption by predators obtained from the GLMMs, which were more analytical in nature. We provided the detailed model results in Table 1, 2, and 3.

**Comment 35** > L. 238: Where are the analyses for this statement?

**Response 35** > This statement was based on the patterns of pest consumption by predators across the three study years in Fig. 2, which shows that pest consumption exhibited similar increasing trends over the rice growing season in all three years).

**Comment 36** > Fig. 2: No measure of variance?

**Response 36** > We have now added the error bars to Fig. 2.

**Comment 37** > L. 285: You cannot state that the spiders become more specialist as you do not include the different group abundances. They might be eating the same proportionally to what is in the field. Rephrase manuscript title accordingly

**Response 37** > We agree that the dietary patterns of generalist predators might reflect what was available in the field. In fact, our beta regression models did not reveal a significant effects of rice herbivore abundance on pest consumption, indicating that those predators might be feeding on more rice pests in their diets than what they would have consumed by random foraging (if the predators had been feeding proportionally to the prey abundance in the field, the models should have revealed a significant effect of rice herbivore abundance on pest consumption). Regardless of the underlying mechanisms for their feeding patterns, these predators did consume high proportions of rice herbivores later in the rice growing season. Therefore, from a “functional” perspective, they acted as specialists of rice pests at late crop stages. We have now modified the title to be more specific about our results: “Generalist predators function as pest specialists at late crop stages: examining the diet composition of spiders and ladybeetles over rice growing season”.

**Reviewer 3's comments**

**Comment 1** >

This study aims to analyse the diet composition of spiders and ladybeetles over the growth season in rice crops. The authors used stable isotope analysis and Bayesian mixing models to identify predator preferences for pests and other herbivores over 3 years of study. In addition, the study intends to cover different gaps of knowledge related to the effect of biotic and abiotic factors (cropping regime, abundance of pests, and complexity of surrounding vegetation) on predator diet composition.

The manuscript is well written and the ideas set out clearly and concisely. I believe that the topic is timely and necessary to elucidate whether generalist predators can be considered natural enemies in crops within the framework of biological pest control. However, the authors focus on highlighting the potential benefits that the presence of generalist predators can bring to pest control in the crop (which I agree with) but it is not until late in the discussion that they refer to feeding behaviours such as intraguild predation, very common in predators such as spiders, which can have a negative effect on biological pest control. In fact, this is the main problem I find with both the sample collection design and the Bayesian mixing model analysis. The authors have focused on analysing the diet composition of predators based on the study of the isotopic content of different herbivores present in the rice crop. However, Bayesian mixing models are very sensitive to missing resources and, considering the great diversity of resources available to spiders, it would be necessary to include other potential non-herbivore prey in the analysis. On the other hand, they use the method proposed by Caut et al. (2009) to calculate TDFs but this protocol was subsequently criticised in several publications and is therefore not the most suitable to be used. In my opinion these are important changes that should be resolved before publishing the manuscript.

**Response 1** >

> We indeed have three sources and non-herbivore prey items in the mixing model

> We originally considered including predator as both consumer and prey sources to account for IGP, but we contact the package author Brian Stock and he replied that this is currently not implemented in the package function and doing do is not recommended

*Key words*

**Comment 2** >

Please remove the keyword “generalist predators” because it is included in the title.

**Response 2** >

*Introduction*

**Comment 3** >

The introduction is concise and clearly explains the gaps in knowledge of generalist natural enemies as biological control agents and why it is important to fill these gaps in order to increase the importance of generalist predators in biological pest control programmes. However, I believe that the authors do not expose to an adequate extent the potential drawback of intraguild predation and the impact that using generalist predators, such as spiders, can have on other natural enemy populations. There are recent articles that claim for further studies that deeply investigate the pros and cons of spider as natural enemies in crops and, despite I understand that it is not the aim of the manuscript, I consider that it would be good to make a reference to this issue.

For instance, Hanbäck et al. (2021) found that an important part of the diet of several spider families was covered by other natural enemies in apple orchards. In addition, Mezofi et al. (2020) showed that the beneficial provided by arboreal spiders as predators of aphids in apple crops is reduced by their high levels of intraguild predation and by a propensity to switch from pests to alternative prey. Saqib et al. (2021) demonstrated the great dietary spectrum of different spider families in Brassica vegetable orchards. Authors highlight the complexity of these predator networks but found some preferences and biological control potential of particular spider taxa.

Hambäck, P. A., Cirtwill, A. R., García, D., Grudzinska-Sterno, M., Miñarro, M., Tasin, M., ... & Samnegård, U. (2021). More intraguild prey than pest species in arachnid diets may compromise biological control in apple orchards. Basic and Applied Ecology, 57, 1-13.

Mezőfi, L., Markó, G., Nagy, C., Korányi, D., & Markó, V. (2020). Beyond polyphagy and opportunism: natural prey of hunting spiders in the canopy of apple trees. PeerJ, 8, e9334.

Saqib, H. S. A., Liang, P., You, M., & Gurr, G. M. (2021). Molecular gut content analysis indicates the inter‐and intra‐guild predation patterns of spiders in conventionally managed vegetable fields. Ecology and Evolution, 11(14), 9543-9552.

**Response 3** >

> Discuss IGP in depth in the discussion section and add the current research on IGP and spiders as pest control agents

**Comment 4** >

L 57-60: Authors emphasize the increasing importance of generalist predators in biological pest control. I agree with authors but it would be important to highlight that in the case of spiders, there are many misgivings about its efficacy as a biological agent due to its cannibalistic behaviour and its ability to feed on other natural enemies present in the crop.

The list of references would be enriched by including recent articles in which spiders have been experimentally identified as potential natural enemies of crop pests:

Morente, M., & Ruano, F. (2022). Understanding the trophic relationships amongst arthropods in olive grove by δN15 and δC13 stable isotope analysis. Journal of Applied Entomology, 146(4), 372-384.

Mezőfi, L., Markó, G., Nagy, C., Korányi, D., & Markó, V. (2020). Beyond polyphagy and opportunism: natural prey of hunting spiders in the canopy of apple trees. PeerJ, 8, e9334.

Cuff, J. P., Tercel, M. P., Drake, L. E., Vaughan, I. P., Bell, J. R., Orozco‐terWengel, P., ... & Symondson, W. O. (2022). Density‐independent prey choice, taxonomy, life history, and web characteristics determine the diet and biocontrol potential of spiders (Linyphiidae and Lycosidae) in cereal crops. Environmental DNA, 4(3), 549-564.

**Response 4** >

> Update the references

**Comment 5** >

L 72-76: The same as above. It is important to consider not only the facility of generalist predators to change their preys but which is the function of these preys in the crop.

**Response 5** >

**Comment 6** >

L 113: Please change: “regardless of the year”

**Response 6** >

*Materials and methods*

**Comment 7** >

L 137: Please add a space between 1.5 and m.

**Response 7** >

**Comment 8** >

L 147-149: Could you be more explicit, how many capsules did you use per taxon (e.g. range, mean number or total number)? I suggest adding the number of capsules in Table S1.

**Response 8** >

> Add the number of experimental units at different levels

**Comment 9** >

L 175-178: I acknowledge the enormous work done by the authors in collecting and analysing a large number of herbivore taxa over several years and in different types of cropping regimes. However, as I have pointed out before, in the case of spiders I am concerned about the effect of intraguild predation and cannibalism on the results of the Bayesian mixing model. Mixing models are sensitive to missing sources (Phillips et al. 2014) and in the case of spiders, I consider it mandatory to include the stable isotope signature of prey other than the herbivore (spiders and other non-spider predators) that might be important in the diet composition of predators mainly in those seasons when pests and other herbivores are scarce. I recommend including in the analysis those predators that show a stable isotopic signature that fits the mixing polygon defined by the sources (in the C-N graph) and that have been previously identified as prey of the predators studied in the literature.

Phillips, D. L., Inger, R., Bearhop, S., Jackson, A. L., Moore, J. W., Parnell, A. C., ... & Ward, E. J. (2014). Best practices for use of stable isotope mixing models in food-web studies. Canadian Journal of Zoology, 92(10), 823-835.

**Response 9** >

> We have three prey sources in the mixing model, and we have two biotracers, which is the ideal situation for mixing model estimation

> We originally considered including predators as a fourth prey source to estimate IGP, but having the same isotope data as both consumer and source in the model is not recommended by the author (personal communication)

> Add a stable isotope biplot to show the TPF-corrected raw predator points as well as their mean and SD in the convex hull formed by the three prey sources (polygon). This also shows the appropriateness of the mixing model as the predator data lie within the polygon. If most points lie within the polygon, then IGP might not be operating at a high degree.

> We also have other alternative prey to reduce the risk of IGP, and also predator abundance is not that high compared to prey sources (high prey abundance so no need to consume each other), therefore IGP may not be important.

**Comment 10** >

L 185-186: Caut et al. (2009) proposed that TDFs vary systematically based on the isotopic values of the diet, but this conclusion has been criticized for the lack of a theoretical and mechanistic basis and also because of mathematical artefacts and experimental biases (Auerswald et al. 2010; Perga and Grey 2010; Codron et al. 2012). Thus, I’m afraid that it is probably not the best option to use for calculating TDFs.

TDFs are one of the most uncertain factors in order to analyse organism’s diet by isotopic analyses. Currently, the best proposed way to estimate TDFs is to conduct controlled studies of consumer-diet N15 and C13 enrichment by isolating the predators of interest with their main prey and analysing their isotopic content at various times after it has been fed. However, I consider that this method is restricted to very specific studies and is not feasible for field studies with generalist predators. In this case, you can search the literature for TDFs previously used for your taxa of interest. If this option is not possible, the third (and least reliable) way is to use pre-established TDFs for predatory insects and spiders (e.g. McCutchan et al., 2003, Vanderklift & Ponsard, 2003).

Auerswald, K., Wittmer, M. H., Zazzo, A., Schäufele, R., & Schnyder, H. (2010). Biases in the analysis of stable isotope discrimination in food webs. Journal of Applied Ecology, 47(4), 936-941.

Perga, M. E., & Grey, J. (2010). Laboratory measures of isotope discrimination factors: comments on Caut, Angulo & Courchamp (2008, 2009). Journal of Applied Ecology, 47(4), 942-947.

Codron, D., Sponheimer, M., Codron, J., Newton, I., Lanham, J. L., & Clauss, M. (2012). The confounding effects of source isotopic heterogeneity on consumer–diet and tissue–tissue stable isotope relationships. Oecologia, 169, 939-953.

McCutchan Jr, J. H., Lewis Jr, W. M., Kendall, C., & McGrath, C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos, 102(2), 378-390.

Vanderklift, M. A., & Ponsard, S. (2003). Sources of variation in consumer-diet δ 15 N enrichment: a meta-analysis. Oecologia, 136, 169-182.

**Response 10** >

> Provide a table of the DDDF, pre-established TDFs, as well as TDFs for spiders or arthropod predators on different arthropod prey

> Do a sensitive analysis by using different TDFs and provide a summary table of the results

> The advantage of using DDDF is that it allows for different TDFs for different prey items as opposed to a fixed TDF. In fact, pre-established TDFs have also received much criticism for generating inaccurate results (citations)

*Results*

**Comment 11** >

L 217-233: Results should be left pending to new analyses including other potential preys and using more adequate TDFs.

**Response 11** >

> Show the results of both DDDF and TDFs and see if they differ

**Comment 12** >

It would be desirable to add the 15N vs. 13C biplot including the convex hull defined by the sources and the position of predators used in Bayesian mixing model.

**Response 12** >

> Add a SI biplot in to the supplementary materials

**Comment 13** >

L 235-245: This is an interesting result that could point to a promising role of GAPs as natural enemies in rice crops, but it would be interesting to see whether these changes in consumption rate over the season are related to a decrease in pest abundance in the crop and, if there is an effect, to test the effect of the cropping regime. That is, beyond a quantitative increase in pest consumption, do GAPs have a positive effect on reducing pest populations over time, and does the cropping regime affect the role of GAPs as natural enemies? I understand that this is not the aim of the manuscript but, if you have pest abundance data, a first approach to the role of GAPs in pest control could take your results a step further.

**Response 13** >

> Show the abundance of pest population and predator population over the season in the three years in organic and conventional farms

> Yes, the pest abundance will be directly related to pest control by predators. Although we did not manipulate predator abundance (e.g., removal of predators) to see the impacts of predators on pest, we do show that they consume high proportion of these pests, providing indirect evidence for their pest regulation potential

*Discussion*

**Comment 14** >

L 282: It is very honest that you comment that intraguild predation and pest suppression may be two potential caveats in your study. However, this is the first question that arises for the reader when reading the introduction, so I think you should include the importance of these two factors in terms of biological pest control at the outset to put the reader in context.

**Response 14** >

> Mention IGP in the introduction and discussion

> We don’t want to distract readers in the beginning and we did not focus on IGP and so we only briefly talk about IGP and we address this issue in depth in the discussion.

**Comment 15** >

L 285: I understand that authors interpret that at the end of the season, of all the herbivore sources they have analysed, the most consumed by both predators are the pests. This is an interesting result that would denote a certain preference for pests. However, considering the need to include other prey non-herbivore in the Bayesian mixing model, and review the TDFs used in the analysis, this statement should be left pending new results.

**Response 15** >

**Comment 16** >

L 337-342: I consider that this is an unexpected result and that it would be interesting to investigate why they consume more pests in conventional crops. Authors cannot assert that there is pest specialisation by generalist predators if they do not know the density of prey taxa in the crop. Perhaps they only feed on pests because that is all that is available. A simple way to do this would be to compare the densities of herbivores (the same ones that have been included in the stable isotope analysis) in the two crops and thus be able to resolve some of the reasons why this may be happening.

On the other hand, one of the main objectives of new agro-environmental schemes is to reduce the application of insecticides and promote biological pest control in crops. Therefore, although it is important to highlight the potential role of GAPs in conventional crops, the authors should indicate that, as a future direction, it would be crucial to know the role of GAPs in organic crops both in terms of efficiency in control of pests (since, as indicated in point 1, they can easily change prey in environments with high species diversity) and their relationship with other natural enemies.

**Response 16** >

> Good suggestion and compare the rice herbivore abundance between organic and conventional farms

> Also the role of GAPs in organic farms needs further research

**Comment 17** >

L 352-354: Just a comment that I assume the authors will have taken into account and which could be commented on in the discussion section. The surrounding habitat may be affecting in terms of recruitment of individuals. That is, it has been described that natural or semi-natural vegetation surrounding the main crop may act as a refuge for natural enemies when pests are scarce by favouring the migration of natural enemies into the crop when herbivore population densities increase throughout the season. Thus, although the results showed no effect on predator diet composition, this effect could be essential in terms of pest control efficiency.

**Response 17** >

> We agree that although landscape factor does not affect per capita interaction strength, it can potentially alter predator-prey population dynamics by providing spatial refuge, thereby influencing pest control efficacy by GAPs. We have added this in the discussion

> In the discussion, even we did not show the effect on diet composition, but vegetation can affect other aspects of predator prey interactions and migration and dispersal in other systems.

*Appendix A*

**Comment 18** >

Table S3: It would be interesting to separate the abundance data by cropping regime and by the classification of guilds done in the manuscript: rice herbivores, tourist herbivores and detritivore.

**Response 18** >

> Modify the table as suggested

**Comment 19** >

L 45: “Three years of study”

**Response 19** >