COMMENTS FROM ASSOCIATE EDITOR: Dr Bret Elderd  
  
Associate Editor  
Comments to the Author:  
I would like to thank the authors for submitting an interesting manuscript examining the potential biocontrol benefits of generalist predators in rice fields.  Like both of the reviewers, I felt that the authors needed to have additional data sources (preferably beyond their survey data) to support their rather strong conclusions in the manuscript.  The study does a nice job of showing that generalist predators will shift their diets based on the frequency of prey in a rice field and that diet composition changes as the prey base changes over time.  However, there is no indication that the generalist predators have any effect on the prey population or on rice yield.  Both of these metrics would seem to be of primary importance when considering whether or not a particular group of predators are an important part of any biocontrol effort.  For instance, if you spray insecticide on the conventional farms that also knock back predator populations, predators could have no effect on the prey population even though the surviving generalist predators are tracking the herbivores in the systems. As one of the reviewers points out, what about predator abundance? Thus, the current analysis seems to be the first step in answering the questions asked.

> GC’s Response:

I totally agree that to fully understand the biocontrol of predators, we need to know not only their diet patters (per capita consumption) but also their abundances. The former is the functional response and the later is the numerical response, both of which are important aspects of the trophic interactions. I think we know this issue and we did talk about this during our manuscript discussions. In fact, we mentioned in our manuscript that our study provides empirical evidence of strong “per capita consumption”, and we also acknowledged in the limitation part that we did not examine the variation in predator abundance, which may also affect their total biocontrol effectiveness.

If necessary, I think we can use our own survey data to show the temporal change in the relative abundance of predators. Alternatively, we can ask Dr. 蔡 for their data. (In fact, I have already taken a look at the relative abundance of predators in our 2018 survey. But the results did not seem to fit in our story.)

Predator.Abd.tiff

Besides the above and the comments by the reviewers that should be taken into consideration.  I also have a couple of additional comments.  1) In their use of the k-means clustering algorithm, how did the authors decide on k = 3?  Was this solely based on the Dominik et al. 2018 study cited in the manuscript?  Why not explicitly test whether or not k = 3 best fits the data?  Maybe, the rice fields used in this study are different in some way from those used in other studies or maybe they are the same.  Either way, a relatively quick analysis of the data could alleviate this concern.  2) In the study design, the authors pick paired farms -- one conventional and one organic.  In their analysis, the paired sampling pattern is never taken into account.  Shouldn't it be included in the analysis? 3) For the analysis of the ''Predators' trophic niches'', the authors give a mean distance-to-centroid measurement to come to a rather strong conclusion about trophic niche breadth.  The means seem rather close together and there is no indication of what the variability about the mean may be.  The variability should be calculated and included in the manuscript.  Then, the appropriate conclusions should be drawn from the mean and the variability.  
  
> GC’s Response:

1) Why chose k = 3 for the cluster analysis? There are two main reasons for this decision. First, the main purpose for cluster analysis is to group families into distinct sources so that the mixing model can estimate the relative contribution of these sources to predator’s diet. For mixing model to work, the number of sources should be no more than the number of tracers (isotopes) + 1, in our case 3. Of course, some studies did have more sources than tracers, but the uncertainties and errors associated with the estimates will largely increase.

Second, the assignment of guilds is itself an arbitrary decision. There is no standard way to do it. It depends primarily on the questions of interest and the goal of the study. Again, we did cluster analysis mainly because we would like to obtain distinct source groups to run the mixing model. Our goal is not to identify how many true “guilds” *per se* there are in the farm. So as the editor pointed out, we can of course easily test whether k = 3 is the best using some statistical criteria, but I think this is not necessary based on our purpose of doing this cluster analysis.

In conclusion, I think k = 3 would be the most appropriate number of cluster considering both the statistical aspect (sources for the mixing model) and the ecological aspect (three main guilds relevant to predators as suggested by Dominik et al. (2018)).

2) This is actually an interesting question that we did not really think about before. The main purpose of using paired farms is to filter out the confounding effects of surrounding landscapes and environmental factors. So if we analyze our data in this way, the statistical power will probably increase (i.e., more likely to detect small differences).

To do so, we can simply calculate the differences in rice herbivore consumption for each organic-conventional farm pair, and test whether the difference is significantly different from zero across all replicate pairs (This is essentially a paired t-test!).

However, even if we pooled all organic/conventional farms and analyzed them together, our results still indicated significant differences in the consumption of rice herbivores between farm types. So I think if we analyze our data using paired samples, our main conclusion should still remain unchanged.

3) I agree with the editor’s concern that the variability of the mean distance-to-centroid may be important yet overlooked in our analysis. In fact, we based our statement only on the mean distance-to-centroid between organic and conventional farms, but did not statistically compare them. This problem is actually easy to fix. Below is a summary of the additional analysis:

|  |  |  |
| --- | --- | --- |
|  | Mean (SD) (‰) | *n* |
| Organic | 2.63a (1.38) | 52 |
| Conventional | 2.13b (1.05) | 51 |

(\*Welch two sample *t*-test, *t95* = 2.10, *P* = 0.04)

REVIEWERS' COMMENTS  
(If no comments are included please see note below)  
  
Reviewer: 1  
  
REVIEWER REPORT FOR THE AUTHORS  
This manuscript presents a study of prey composition of (unspecified) generalist predators at organic and conventional rice farms using stable isotope analyses at different crop stages. With their analyses the authors can estimate the proportion of rice herbivores, other herbivores and detrivores that the predators have feed upon. In itself this is interesting information and a step towards better understanding biological control of rice pests. However, as shown already by the title, the paper claims to have investigated biocontrol efficacy which is not correct. The abundance of predators was not taken into account and there was no independent measure of impact of the predators on pest populations. What was studied is how the proportion of prey in the guts of generalist predators varies with crop stage, relative abundance of different prey types and with type of management. The manuscript must be rewritten to reflect this.  
  
> GC’s response:

Again I agree with this reviewer’s concern, which is also pointed out by the associated editor. Basically, what our results revealed were the trophic dynamics of arthropod communities in rice farms. We were examining the trophic ecology of predator-prey interactions rather than the biocontrol effects of predators. The potential for predators as biocontrol agents is a selling point of our study, though I feel that we might have a bit over-stated it. So of course we can relate our result to biocontrol and agricultural application, yet we should be a bit more conservative about this. Our results did not seem to provide strong support to our claim of “high biocontrol efficacy”. (Or it may depend on how we define “biocontrol”: reduction in pest populations? or high per capita pest consumption?)

Overall, if we need to rewrite the manuscript, I think we can interpret and discuss our results in a more “ecological” manner instead of “application” manner. I would suggest that our main story revolve around the “trophic dynamics of arthropod communities”, and the manuscript can then be structured accordingly and written in a way to tell this story. Finally, we can link it to agricultural management and application to put our study in a larger context.

Specific comments:  
  
1)      Title. See above. Please change to reflect what is studied, i.e., predator diets.

> GC’s response: a potential title could be “*Pest consumption by arthropod generalist predators increases with crop stage in organic and conventional farms*”.

2)      In general I find the introduction unbalanced. It makes it sound like 1) the value of generalist predators is unclear, but that 2) stable isotope analyses will clarify their role. I would like to see a more balanced discussion of what is actually known about generalist predators and how stable isotopes can help. What are the pros and cons with this method compared with others? There are lots of other studies who have used stable isotope, including comparing organic and conventional systems (e.g. Birkhofer et al 2011 Agricultural and Forest Entomol). What did they find, and what could they not explain with that method?

> GC’s response:

I think we can structure our introduction in this way:

* Importance of arthropod generalist predators in agro-ecosystems >
* Many factors can affect predators’ role: population dynamics of pest and alternative prey >
* Understanding (and quantifying) their trophic dynamics (temporal variations in predator-prey interactions) would be a critical step to evaluate predators’ biocontrol potential >
* Our stable isotope mixing model provides an ideal approach to achieving this goal (including the strength of this method and the comparisons with other studies that also used stable isotopes but did not quantify predator-prey trophic interactions.)

3)      Line 27-28. I suggest changing to ‘… and conventional farms shifted trophic niches over the crop cycle and consumed increasing proportions of rice herbivores…’

> GC’s response: maybe use “over the crop stages” instead of “crop cycle” since we use “crop stage” in our main text.

4)      Line 65-66 Why specifically late in the season? A key advantage in some systems is that they can be abundant early in the season before the pests have arrived. This is for example the case for aphids in spring-sown barley (e.g. Östman et al. 2003 Ecol Econ).

> GC’s response: I think the reviewer might have mistaken what we mean by “later in the season”. Here, we are saying that predators can suppress “pest populations” that build up “later in the season”, rather than saying that predators can “suppress pests” later in the season and are important mainly at that period (but not early season).

5)      Line 86-88. Why not?

> GC’s response: Experimental manipulations (e.g., cage experiments) usually have fixed number of predators in the settings, which may only represent a narrow range of their natural abundance in the field. Also, in the cage experiments, predators and prey are confined in a fixed space and are not allowed to move freely. This may interfere with the natural behavior of predators and prey and potentially alter their interactions.

6)      Line 88-90. I certainly see a place for studies of stable isotopes. It can give an idea of long term broader feeding patterns, but I see it more as a complement to exclusion studies or detection of DNA in the guts. So I would rather say you are providing complementing information compared to other approaches.

> GC’s response:

Sure, besides stable isotope approach, there are also other methods that can examine predator trophic dynamics, and gut content analysis is one of the common examples. So I agree that our study “complements” previous studies on generalist predators in agro-ecosystems. However, in our manuscript, we say that our stable isotope approach can help clarify predators’ role in agro-ecosystems, but we did not say that our approach is the “only” or “main” way to achieve this, while other methods cannot.

By the way, the problem with gut content analysis is that it only provides a “snapshop” of what the predator has recently consumed, but does not give us a broad picture of their diet. This is why our study aims at using stable isotopes to study predators’ trophic ecology.

7)      Line 93. Change to ‘…over the crop cycle.’

> GC’s response: I see no reason why we should use “crop cycle” instead of “crop stage”. For me, they mean the same thing. Not sure if there is any subtle difference in the accurate usage of these two words.

8)      Line 107-108. Could you provide some more information about how the organic and conventional farms were selected and how organic differs from conventional management in the area? How did you ensure that they mainly differed in terms of farming system? How were they located in the landscape, how far from each other were they located? Size of farms/field etc? How long had the organic farms been organic? In what way did the organic farms differ from the conventional ones? How much were the conventional farms sprayed for example?

> GC’s response: I agree. We should provide more information about our study sites.

9)      Line 196 – 200. Please explicitly explain what this shift in diet position means.

> GC’s response: Based on the isotope signatures of prey sources, the temporal shifts in predators’ trophic niches from upper right to lower left implies that they switched their diet from relatively more detritivores/tourist herbivores to more rice herbivores. This could reflect the change in background prey densities, or may be due to changes in predator’s prey preference.

10)     Line 221. Again, you are not investigating biocontrol efficacy in this paper. You’re the feeding niches of predators. To assess biocontrol efficacy you need to know how many predators are available in comparison to prey and you need an independent estimate of pest population reduction.

> GC’s response: I agree. We may want to rewrite it.

11)     Line 228-232. It is confusing to write ‘consumed higher proportions of rice herbivores’ etc. This makes it sound like you studied how large proportion of the population of rice herbivores that was predated, but this is not the case. You only studied how large proportion of the predator diet that comprised of rice herbivores.

> GC’s response: maybe “higher proportions of rice herbivores in their diet” will be clearer so that readers can understand our “proportion” refers to the proportion of diet rather than the proportion of prey populations in the field.

12)     Line 244-246. I disagree with this. It could simply be a consequence of increasing populations of rice pests over time.

> GC’s response: Yes, higher consumption itself does not necessarily mean a greater biocontrol effect. So instead of saying “increasing biocontrol efficacy of predators”, we can say “increasing per capita predator control on pests”. (I feel that we should better define “biocontrol efficacy” in our manuscript and make clear the difference between “per capita consumption” and “reduction in pest populations”. These may be quite confusing to the readers.)

13)     Line 253-254. Here you suddenly mention that you have measures of relative abundance of different prey types. I guess this is from the sweep net samples. It is very important that you have these data and I think you should bring this up in the results section and not only in the supplement.

> GC’s response: Since in our study we discussed the importance of prey abundances in affecting predator-prey trophic dynamics, we can of course place this part in our main text and talk more about it.

14)     Line 256-257. I don’t follow this argument.

> GC’s response: I think we may want to rewrite it. A few problems with this statement:

(1) We did not really show the effects of farming practices on generalist predators’ populations.

(2) We did not really show the connection between predator populations in early season and suppression of pest populations in late season.

(3) Again, the term “pest control” here is vague.

15)     Line 294-296. This could simply be a reflection of higher proportional abundances of rice pests on conventional farms as seen in Appendix S1: Figure S2.

> GC’s response:

Of course this could be the case. Nonetheless, we found that predators in conventional farms were able to keep track on the pest populations and consume high proportions of pests in their diet accordingly. It is often thought that generalist predators in conventional farms might not be as important in organic farms, as pest control can be mainly achieved by chemical application, yet our study actually showed that these predators can also play key functional role in predator-pest trophic interactions in conventional farms.

16)     Line 309-311. Please refer to the other similar studies here. How do the results of their studies compare to yours?

> GC’s response: I think our claim to be the first to “quantify predators’ diet” is not an exaggeration. There have been several previous studies that used stable isotopes to examine predator-prey interactions in agro-ecosystems. However, most of these studies only made inferences about trophic links by comparing the raw isotope signatures of different guilds. On the other hand, our study not only presented qualitative view of predator-prey trophic interactions (i.e., predators’ trophic niches), but also went a step further by providing quantitative estimates of their interactions (i.e., predators’ dietary proportions), which I think is the main distinction from other studies.

17)     Line 318-324. Thank you for highlighting this. The density of different predator species is critical for biological control. You studied prey choice of predators, not their biological control efficacy.

> GC’s response: Yes, both per capita effect (functional response) and abundance (numerical response) are important for predators’ biocontrol. (I think we should make this point clear in our introduction instead of mentioning it in the last part of the manuscript, so that the readers can have an idea in their mind and will not get confused.)

18)     Line 334. Again I disagree. You show that consumption increased with increasing rice herbivore abundances. There is no evidence of increased biocontrol effect.  
  
> GC’s response: We may need to revise our conclusions (and in fact the overall thesis of this study).

Reviewer: 2  
  
REVIEWER REPORT FOR THE AUTHORS  
Hsu et al present an interesting evaluation of trophic position, using stable isotope analysis, among a community of generalist predators in rice. A clear strength of the work is that it was conducted in production rice fields under two systems, organic and conventional. Farms were paired between farming systems to control, to a degree, for the influence of surrounding landscapes. The key “take home message” from the work was that predators in conventional fields exhibited isotopic signatures consistent with a higher proportion of rice-herbivore prey than were consumed by predators in organic fields, and that all predators consumed more pest through the cropping cycle. The methods (sample storage, R packages used, etc) appear appropriate, and the study was conducted in a globally-important crop under realistic, field conditions. While the work has some clear strengths, I also had a few specific concerns:  
  
1) Worries about the role of generalists in biological control are not limited to concern that they will feed on detritus-feeding rather than herbivorous prey, but also that they will feed on other predators. Of course, feeding as a “higher order” predator can also change isotopic signatures. That being said, since the trend is moving to lower d15N and d13C values later in the season, it implies that they are feeding on lower trophic levels as the cropping cycle unfolds. Nonetheless, I worry that failing to consider intraguild predation when assessing trophic position weakens what the authors can say about the ecology of this system.

> GC’s response: Thanks for pointing out this important interaction. In fact, studies have shown that intraguild predation is quite common in the agro-ecosystems, and these antagonistic interactions could largely reduce their pest suppression potential. Yet, so far there is no way to quantify intraguild predation using stable isotope mixing model (of course you can treat predators themselves as a source, but this may not provide reliable estimates since mixing models require distinct consumer and source isotope signatures).In addition, if the predators do exhibit strong intraguild predation, then their isotope signatures (especially N) should be higher than they would have been without intraguild predation, and this increase in N will be reflected in our trophic niche and mixing model. Thus, even though we did not explicitly account for intraguild predation, our study has in part implicitly incorporated its effect.

2) Looking at Figure 2 in combination with Supplemental figure S1, it looks like quite a few predators are falling below all the prey groups in both d15N and d13C. This raises the possibility that there might be another source not being accounted for in this system, and that the only reason rice herbivores are being calculated as a high % of the estimated diet is that it's simply the closest out of the listed sources. This is especially relevant because MixSIAR doesn't have an option to allow for an unknown source unlike a similar package, SIAR.  
  
> GC’s response:

After correcting for trophic discrimination factors (TDFs), the mean of all predators did lie within the polygon bounded by the three sources, which justified the use of mixing model to estimate the proportional contribution of each source. Moreover, all models require certain assumptions. Stable isotope mixing models are no exception to this, and in this study we made the assumption that the three prey guilds were the main sources of predators.

When looking at individual predators, we can see some variations in predator’s isotope signatures within each farm-stage combination (Figure 2.). One possible explanation is that these predators are generalists, so they may show individual variations in resource use. If some individuals do feed more on other resources not identified in our study, it could result in some individuals having isotope signatures lower than that of the prey sources in our study.

However, it is unlikely that we have missed some “important” sources in our system, based on both the literature as well as the isotope values. Since rice herbivores were the main primary producers in our system, their isotope signatures should in theory be the lowest among consumers, and so I don’t think there would be another “important” sources that have similar or even lower isotope values than rice herbivores.

In addition, our goal is to examine the overall dietary patterns of predators, so even if there were individual variations as these individuals might use some unknown resources, we still provided an overall picture of the diet of this trophic guild.

For the R package, I think there are both strengths and weaknesses regarding MixSIAR and SIAR. We chose MixSIAR over SIAR mainly because MixSIAR incorporates more model options, making it more versatile and flexible for the users.

3) The number (N) of the predators involved in the calculations was unclear. Of course, the variability of the trophic niche would be impacted by how many individuals it's based on. The authors could correct this by presenting these numbers.

> GC’s response: Thanks for pointing out this important thing. We should definitely report the sample size of predators for each farm type-crop stage combination. I just quickly summarized it in the below table. I think we can present this information in Appendix S1: Table S3 in a new column “Number of predators”.

|  |  |  |
| --- | --- | --- |
| Farm type | Crop stage | Number of predators |
| Organic | Tillering | 27 |
|  | Flowering | 12 |
|  | Ripening | 13 |
| Conventional | Tillering | 28 |
|  | Flowering | 12 |
|  | Ripening | 11 |

4) As the authors note (e.g., lines 318-322), consistent feeding on herbivores does not necessarily mean that predators are contributing to pest control. Including this caveat in the Discussion is important, but I would look carefully at the repeated implications throughout the manuscript that biocontrol is being altered – perhaps these should be toned down. Looking across all of the figures, the differences between the organic and conventional cropping systems seem relatively modest. This heightened my curiosity whether the patterns were sufficiently strong to lead to ecologically-meaningful differences in predator effects in the field.

> GC’s response: As all three editors and reviewers have mentioned, per capita predator consumption alone may not necessarily translate into a higher biocontrol efficacy. So I think this would probably be the main issue we have to fix in our manuscript.

Since our figures mainly show the per capita effect, the difference might not seem that obvious between the two farm types. Yet the total effects also rest on the abundance of predators in the field. Future work would require incorporating these two aspects to examine whether the overall ecological effects of predators differ among farm types.

5) It doesn’t seem fair to say that “quantitative studies regarding the trophic dynamics of generalist predators in agro-ecosystems are lacking”. In fact, the Settle et al. (1996) study cited in this paper is a classic example of predators switching between detritus- versus plant-feeding prey to improve biocontrol of rice pests. Many other studies look at impacts of non-pest prey on biocontrol, or on how intraguild predation interrupts biocontrol. Throughout, I would suggest that the authors work to make a more nuanced case about where their findings fit in the broad literature looking at generalist predator as biocontrol agents.

> GC’s response: The word “lacking” is the crux: By “lacking”, we do not mean there are no such studies, but just few. Settle et al. (1996) is the classic one, and we did bring up this paper several times in our manuscript. I agree that this word is a bit too strong and assertive. Maybe we can rewrite the whole sentence something like this: “Although there have been several quantitative studies on the trophic interactions of generalist predators in agro-ecosystems, it remains unclear how these interactions are affected by temporal dynamics of prey sources, which may hinder our ability to assess their efficacy as biocontrol agents.”

On the other hand, as the reviewer suggested, it is important to really think over how our study fits in with other research, and how it contributes to this field.

6) The authors are lumping some fairly broad groups of predators into single trophic groups, which might pool species with very different feeding positions (e.g., Steffan et al. 2015, Biological Control 91:34-41). In general, the paper would be strengthened with a second, complementary approach beyond stable istotpes to verify that the trophic interactions implied by the isotopes reflect, for example, feeding interactions that are directly observed in these same fields, or impacts on prey seen with experimental predator manipulations.

> GC’s response: Yes, I agree. There are many approaches to the same research questions, and they may offer different angles of views. Complementing these results allow for a broader and thorough picture of the arthropod trophic dynamics in agro-ecosystems. This actually highlights the importance of finding how our study fits in the current knowledge framework and furthers our understandings in this field.

As for the lumping issue, of course the resolution might seem relatively course and we could potentially miss some individual variations. If we would like to compare the difference among different generalist predators, then lumping broad groups of predator species may be inappropriate. However, in this study we are interested in the overall patterns of predator trophic guild, and this justifies pooling different predator families in a single trophic group.