Response to Reviewers’ Comments

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**Manuscript Number**: JZO-08-22-P-196

**Title of Article**: An experimental framework for quantifying the degree of intraguild predation in omnivorous food webs in the field

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Dear Dr. Richelle Tanner,

Thank you for inviting me to submit a revised version of the manuscript. I greatly appreciate the valuable comments and feedback from the reviewers. I have incorporated most of the suggestions and the revision has substantially improved the manuscript. In particular, I have made the following major changes in this revised manuscript:

* Corrected the citation format issue and added several recent articles to the manuscript to better reflect the current status of IGP research.
* Introduced the use of molecular gut content analysis for studying IGP and its potential limitation in the fourth paragraph of the *Introduction* section.
* Discussed the issue of ontogenetic shifts in prey nitrogen isotope signatures and how to address it via stage-specific IGP estimates in the fourth paragraph of the *Applications* section.
* Discussed the issue of mesopredator feeding on alternative prey and how to address it by calibrating the δ15N of top predator in the fifth paragraph of the *Applications* section.
* Discussed the issue of top predator feeding on non-focal prey and suggested several methods to address it in the sixth paragraph of the *Applications* section.

Please also see the following section for my detailed point-by-point responses. All line numbers pertaining to the changes made refer to the revised manuscript.

Sincerely,

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**Associate Editor's Comments to the Author:**  
  
**Comment** > Both reviewers are positive about this manuscript and make important recommendations that will improve its scope and strength. The author might wish to consider a revision of their title, especially in line with the discussions and implications suggested by reviewer 2 (i.e. is their approach only relevant to a three-species food web?). The author might also wish to explore advanced approaches of stable isotope trophic ecology beyond bulk isotopes, such as compound-specific stable isotopes (e.g. essential amino acids) - might this add value to the interpretations that can be made from complicated food webs where not all dietary items are known or sampled? (again, in line with some of reviewer 2's comments)

**Response** >

I would like to thank the two reviewers for their positive attitude towards this manuscript and their constructive comments on the potential limitations of the proposed framework. I have carefully considered the concerns raised by the reviewers and made several changes accordingly (particularly regarding top predator and mesopredator consumption on alternative prey by and cannibalism in top predator). In this regard, I have also modified the title (removed “three-species”) since the revised manuscript now extends beyond only three focal species and considers a broader suite of trophic interactions in omnivorous food webs.

* Compound-specific nitrogen isotope analysis of amino acids (CSIA-AA):

1. Can be used to infer trophic positions when not all prey items are known > To estimate the TP of an animal, BSIA always requires isotope data for both the animal and its diet(s). CSIA-AA does not need to characterize any diet source data to estimate the TP of the focal animal, because the TP value is represented as an offset between the d15N values of glutamic acid (d15NGlu), which increase considerably with TL, and phenylalanine (d15NPhe), which remain unchanged during trophic transfer, in the animal.
2. The values are based on energy transfer and biomass assimilation, not the “number” of prey items consumed
3. I agree that although CSIA-AA is still a relatively new technique in ecology, and associated theories and models and methodological details are still under development, but it can complement the current framework
4. The CSIA-AA methodology is still in its infancy, and there is a vast expanse of research field in trophic ecology to be addressed with this promising tool.

**Reviewer 1's Comments to the Author:**

**Comment** > Intraguild predation (IGP) is very common in arthropods. However, due to too many uncertain environmental factors and technical methods, it is difficult to quantify IGP. In this study, the control feeding experiment and stable isotope analysis of field samples were combined to evaluate the degree of IGP in a three-species omnivorous food web (top predator + mesopredator + shared prey). The experimental design of this study is reasonable, and the stable isotope analysis technology used is relatively mature and reliable, which can solve the problem of quantifying IGP to a certain extent.

**Response** > Thanks for the positive comments on my manuscript.

Enumerated Concerns:

**Comment** > Manuscripts should quote more literatures published in the past five years, while the current cited literatures have been published for a long time, which does not reflect the cutting-edge research.

**Response** > I have added several recent articles to the manuscript as described below:

* Wang, S., Brose, U. & Gravel, D. (2019). Intraguild predation enhances biodiversity and functioning in complex food webs. Ecology, 100, e02616. (Line XXX)

Using a modeling approach, this study shows that IGP could enhance biodiversity and ecosystem functioning (e.g., total biomass or primary production).

* Hagler, J. R., Casey, M. T. & Machtley, S. A. (2020). A procedure for pinpointing cannibalism, intraguild predation, and life stage-specific feeding events. BioControl, 65, 297-304. (Line XXX)

This article discusses the use of molecular methods and immunological techniques to study IGP.

* Snyder, G. B., Smith, O. M., Chapman, E. G., Crossley, M. S., Crowder, D. W., Fu, Z., Harwood, J. D., Jensen, A. S., Krey, K. L. & Lynch, C. A. (2022). Alternative prey mediate intraguild predation in the open field. Pest Management Science, 78, 3939-3946. (Line XXX)

This study applies molecular gut content analysis to estimate the probability of IGP on mesopredator *Geocoris sp*. by the top predator *Nabis sp*.

* 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, 9543-9552. (Line XXX)

This is a recent example study that quantifies the strength of IGP based on the proportions of prey DNA reads in top predator’s gut contents.

* Macías-Hernández, N., Athey, K., Tonzo, V., Wangensteen, O. S., Arnedo, M. & Harwood, J. D. (2018). Molecular gut content analysis of different spider body parts. Plos One, 13, e0196589. (Line XXX)

This study shows that the detectability of prey DNA sequences varies among different parts of the spider’s gastrointestinal tract.

* Michalko, R., Birkhofer, K. & Pekár, S. (2022). Interaction between hunting strategy, habitat type and stratum drive intraguild predation and cannibalism. Oikos, 2022, e08662. (Line XXX)

This study examines how habitat type (vegetation vs. ground) affect intraguild interactions among spider mesopredators and top predators.

**Comment** > Please check the description format of references in and after the text. The format is inaccurate in some places.

**Response** > Thanks for pointing out the citation format mistakes. I have corrected the citation style template in my EndNote library and made the following changes to the in-text citations (“original” to “revised”):

* “GagnonHeimpel & Brodeur 2011” to “Gagnon et al., 2011” (Line XXX)
* “PolisMyers & Holt 1989” to “Polis et al., 1989” (Line XXX)
* “UiterwaalDell & DeLong 2019” to “Uiterwaal et al., 2019” (Line XXX)
* “WiseMoldenhauer & Halaj 2006” to “Wise et al., 2006” (Line XXX)
* “RickersLangel and Scheu (2006)” to “Rickers et al. (2006)” (Line XXX)
* “CautAngulo & Courchamp 2009” to “Caut et al., 2009” (Line XXX)
* “SchneiderScheu & Brose 2012” to “Schneider et al., 2012” (Line XXX)
* “QuinbyCreighton & Flaherty 2020” to “Quinby et al., 2020” (Line XXX)

**Comment** > In the sentence of collecting samples in the field (L 132 − 134), it is necessary to clarify the type and spatial location of the sampling habitat, or explain the environmental factors in the collection area, so as to eliminate the impact of other different environmental factors as far as possible and improve the accuracy of evaluating the degree of IGP.

**Response** > This is a critical point for accurately quantifying the degree of IGP in the field. The predator and prey samples should be taken under homogeneous conditions to minimize the effects of various environmental factors, and also at an appropriate spatial scale relevant to the research goal. I have revised the fifth paragraph in *The proposed experimental framework* section to incorporate this information. (Line XXX)

“Finally, field samples of the top predator and shared prey are collected for stable isotope analysis under homogeneous site conditions (e.g., similar ambient temperature and vegetation structure) to minimize the potential confounding effects of abiotic and biotic factors, and the spatial scale at which the samples are taken should pertain to the research goal (e.g., various locations within a farm to quantify farm-level IGP, or various plots within a one-hundred-hectare grassland to quantify community-level IGP).”

**Reviewer 2's Comments to the Author:**

**Comment** > The present manuscript propose a method to evaluate the intraguild predation in trophic webs by using the N15 isotopic content of top predators, meso-predators and a shared prey aiming to construct an IGP curve based on controlled feeding trials. The author proposes to use this IGP curve to estimate the degree of intraguild predation in the field. IGP is a problematic issue in trophic web studies when a large number of taxa are included. The author´s proposal is an interesting point of view aiming to facilitate the management of field data but I believe it could be useful for simpler trophic interactions than the example proposed in the manuscript (although is asimple tri-trophic web, spiders have very complex feeding preferences). I am aware of the difficulty of studying arthropod food webs in the field and the advantages and disadvantages of using stable isotopes. I would like to expose several considerations about the proposal in general:

The main problem of the IGP to study trophic interactions in very generalist predators, such as spiders, is the high number of potential prey available in the field (including cannibalism) that could be determinant to establish correct levels of N15 and unapproachable in an experimental trial. In fact, although these generalist taxa belong to agricultural systems (simplified systems), the variety of prey can be very high. The author considers this limitation and recommends collecting large enough field samples of top predator to reflect the overall IGP patterns. However, other limitation comes from those cases where phytophagous insects show high levels of N15. For example, in Lepidoptera, the metamorphosis metabolism results in adult individuals with N15 levels comparable to those of predators (see Tibbets et al. 2008). Predating on such phytophagous insects could lead to a high enrichment in the N15 content of top predators not produced by the IGP. Therefore, I consider that this procedure could be useful for other less complex food webs in which IGP is present but the number of potential prey for top and meso-predators is less varied (Acari or Neuroptera for instance). In line with the same subject, in lines 168-172 the author indicates that it is possible to adjust the N15 signature of top predators that feed on non-focal prey. What is the way to do this calibration without knowing the N15 of these other preys? Please specify the way to calibrate N15 and add references.

As the author rightly points out, this is a promising proposal but one that needs to be refined in the future. Therefore, in order to be able to carry out the necessary experiments to fine-tune this protocol, it would be necessary to set out in more detail its limitations.

Tibbets TM, Wheeless LA, Del Rio CM, 2008. Isotopic enrichment without change in diet: An ontogenetic shift in δ15N during insect metamorphosis. Funct. Ecol. 22, 109–113.

**Response** >

Thanks for pointing out several critical points regarding the implementation of the proposed framework. I appreciate the suggestions and have revised my manuscript accordingly where appropriate. The details are provided in the following sections.

A major concern of the proposed framework is that top predator can feed on alternative non-focal prey items in the field, which may interfere with the interpretation of the N15 signature of top predator. In fact, as stated in the third paragraph of the *Applications* section, the framework is best suited to relatively simple food webs with strong interactions among the focal species, in agreement with the reviewer’s opinion that the framework is useful for less complex food webs in which IGP is present but the number of potential prey for top predator and mesopredator is less varied.

For more complex webs, researchers can first identify the potential non-focal prey in top predator’s diet via field observations or molecular gut content analysis, collect field samples of these prey items and analyze their nitrogen isotope signatures, and adjust the δ15N of top predator by subtracting the δ15N difference between non-focal and the focal shared prey (also see the sixth paragraph of the *Applications* section for more details). Alternatively, researchers can directly include these non-focal prey items in the feeding trials to account for their effects on intraguild interactions and thus the δ15N of top predator. Finally, collecting large field samples of top predator can help better capture the overall IGP patterns.

As the reviewer mentioned, some top predator species (e.g., spiders) may engage in cannibalism, which can alter their δ15N values in the field. In this case, I suggest rearing multiple top predator individuals in the same experimental arena to allow for cannibalism events in the feeding trial. This can better reflect predator-predator interactions in the field to account for their effects on the δ15N of top predator (also see my detailed response to comment X).

Another potential limitation the reviewer pointed out is the nitrogen isotope enrichment from larvae to adults in phytophagous insects due metamorphosis metabolism. Consuming these N15-enriched prey items may lead to a high δ15N of top predator not produced by IGP. To address this issue, researchers can apply the framework using prey individuals at different developmental stages in separate feeding trials to derive stage-specific standard IGP curves; the stage-specific IGP estimates can then be linked together to form the overall IGP pattern (also see the fourth paragraph of the *Applications* section for more details).

As the reviewer commented, it is necessary to consider several potential limitations and further explain the experimental details. I agree with the reviewer’s opinion and have revised the manuscript accordingly for a more thorough discussion of the concerns raised and the ways to address them. Overall, I believe the proposed framework can serve as a useful tool for studying IGP dynamics in the field.

*Abstract*

**Comment** > Line 32: to study.

**Response** > Revised. (Line XXX)

*Introduction*

**Comment** > Line 101: Quinby, Creighton & Flaherty 2020 (add a comma between authors).

**Response** > Thanks for pointing out the mistake. I have changed the original citation “QuinbyCreighton & Flaherty 2020” to “Quinby et al., 2020” (please also see my response to Reviewer1’s comment on the citation format issue).

*The proposed experimental framework*

**Comment** > Line 96-108: Have been the arthropods kept under starving for a time before the experimental trial?

**Response** > Yes, the experimental organisms should be starved for a period of time prior to the feeding trials to avoid potential contamination from the previously-consumed food in their guts. Additionally, field-collected organisms should be starved as well (if possible) to empty their gut contents before stable isotope analysis. I have added these experimental details to *The proposed experimental framework* section. (Line XXX)

“All experimental organisms are starved prior to the feeding trial to avoid potential contamination from their gut contents.”

“All experimental organisms are starved beforehand as in the first feeding trial.”

“If possible, the field-collected organisms should be kept in starvation to empty their guts before preparation for stable isotope analysis.”

**Comment** > Line 108-110: In the case of spiders or other arthropods such as larval green lacewings it would be desirable to consider the cannibalism event in the experiment including individuals from the same species in the proportions of the diet.

**Response** > Thanks for bring up this important point. Yes, cannibalism among top predator individuals should be accounted for in the experimental framework as it could potentially alter the interactions between top predator and shared prey/mesopredator. Therefore, for those top predator species that engage in cannibalism, multiple predator individuals should be reared together in the same experimental arena so that the effects of cannibalism can be better reflected in the nitrogen isotope signatures of top predator. I have discussed this in the sixth paragraph of the *Applications* section. (Line XXX)

**Comment** > Is the meso-predator fed with the shared prey before the assay or is kept under starving for a time? It would be important to include these two treatments in the experimental assay because the gut content of the meso-predator may affect the N15 content of the top predator.

**Response** > The mesopredator used in the second feeding trial should be starved to minimize the potential effects of consumed shared prey in the gut contents on the isotope signatures of the top predator. I have added this experimental detail to *The proposed experimental framework* section (Line XXX) “All experimental organisms are starved beforehand as in the first feeding trial.”. Please also see my response to the previous comment (“Line 96-108: Have been the arthropods kept under starving for a time before the experimental trial?”) for more details on starving the experimental organisms.

**Comment** > Line 120-121: Likewise the top predator, the mesopredator is a generalist spider that can eat other resources different from the shared prey used in the experimental trial. Therefore, the N15 content of mesopredators may vary respect to the individuals used in the experimental trial. It would be necessary to collect mesopredator individuals from the field aiming to know the N15 content in field conditions.

**Response** > Thanks for pointing this out. Yes, I agree that it would be helpful to analyze the nitrogen isotope signature of mesopredator in the field as well since it can feed on prey items other than the shared prey. To account for this variation, one can calculate the average δ15N difference between the field-collected and lab-reared (those feeding entirely on the shared prey) mesopredator individuals and calibrate the δ15N of top predator individuals by subtracting this δ15N difference. I have discussed this in the fifth paragraph of the *Applications* section. (Line XXX)

*Applications*

**Comment** > Line 138: to study.

**Response** > Revised. (Line XXX)