<div align="center">**Response to Reviewers' Comments**</div>

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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:

- 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 estimation 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 $\delta^{15}N$ 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.
- Discussed the potential application of compound-specific nitrogen isotope analysis of amino acids in the proposed framework in the seventh 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 refer to the revised manuscript.

Sincerely,

Gen-Chang Hsu

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Associate Editor's Comments to the Author:

Comment 1 > 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 and cannibalism in top predator). 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.

I would also like to thank the editor for bringing up the advanced isotope technique of compound-specific nitrogen isotope analysis of amino acids (CSIA-AA). I have now briefly discussed the potential application of CSIA-AA in the proposed framework and pointed to several references on this new technique in the seventh paragraph of the "*Applications*" section (Line 204-218):

"Recent advances in compound-specific isotope analysis of amino acids (CSIA-AA) provide a promising tool for studying trophic interactions (see Ishikawa [2018] and McMahon & McCarthy [2016] for more details on the use of CSIA-AA in trophic ecology). A major advantage of CSIA-AA over bulk stable isotope analysis is that it can estimate trophic positions (TP) of consumers even when some of their prey items are unknown to researchers. A potential application of CSIA-AA that may aid in the proposed framework is to analyze and compare the TP of field-collected and lab-reared top predator. Theoretically, if top predator consumes more non-focal prey items in the field, its TP will deviate more from (presumably be lower than) that

of the lab-reared top predator because it incorporates more lower-level biomass into the tissue. In this regard, the amount of deviation can allow researchers to gauge the actual degree of IGP in the field relative to the one estimated via the controlled feeding trial (e.g., a larger deviation in TP may indicate a lower actual degree of IGP in the field compared to the IGP estimated via the feeding trial). Additional measures can be taken to account for the effects of non-focal prey on IGP interactions (see previous paragraph for details)."

Although CSIA-AA is relatively new to ecologists, and relevant theories, analytical models, and methodological details (e.g., trophic discrimination factors for ^{15}N of different amino acids) are currently in development, I agree that it certainly provides a promising tool for better studying IGP and complex food web dynamics.

Reviewer 1's Comments to the Author:

Comment 1 > 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 1 > 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 46)

This modeling work 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 66)

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 68)

This study applies molecular gut content analysis to estimate the probability of IGP on the 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 74)

This is a recent example study that quantifies the strength of IGP based on the proportions of prey DNA reads in the 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 76)

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 160)

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

Comment 2 > 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 44 and 66)
- "PolisMyers & Holt 1989" to "Polis et al., 1989" (Line 44, 46, and 166)
- "UiterwaalDell & DeLong 2019" to "Uiterwaal et al., 2019" (Line 54)
- "WiseMoldenhauer & Halaj 2006" to "Wise et al., 2006" (Line 56)
- "RickersLangel and Scheu (2006)" to "Rickers et al. (2006)" (Line 57)
- "CautAngulo & Courchamp 2009" to "Caut et al., 2009" (Line 63)
- "SchneiderScheu & Brose 2012" to "Schneider et al., 2012" (Line 81)
- "QuinbyCreighton & Flaherty 2020" to "Quinby et al., 2020" (Line 101)

Comment 3 > 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 123-128):

"Finally, field samples of the top predator and shared prey are collected under homogeneous site conditions (e.g., similar ambient temperature and vegetation structure) to minimize the potential confounding effects of abiotic and biotic factors. 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 1 > The present manuscript propose a method to evaluate the intraguild predation in trophic webs by using the N^{15} 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:

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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 N^{15} 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 N^{15}. For example, in Lepidoptera, the metamorphosis metabolism results in adult individuals with N^{15} levels comparable to those of predators (see Tibbets et al. 2008). Predating on such phytophagous insects could lead to a high enrichment in the N^{15} 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 N^{15} of these other preys? Please specify the way to calibrate N^{15} 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 $\delta^{15}N$ 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 the manuscript accordingly where appropriate. The details are provided in the following paragraphs.

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 $\delta^{15}N$ values 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 $\delta^{15}N$ of top predator by subtracting the $\delta^{15}N$ difference between non-focal and the focal shared prey. Alternatively, researchers can directly include these non-focal prey items in the feeding trials to account for their effects on IGP interactions and thus the $\delta^{15}N$ of top predator. Finally, collecting large field samples of top predator can help better capture the overall IGP patterns at the population level (see the sixth paragraph of the *Applications* section for more details).

As the reviewer mentioned, some top predator species (e.g., spiders) may engage in cannibalism, which can alter their $\delta^{15}N$ 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 will better reflect predator-predator interactions in the field and account for their effects on the $\delta^{15}N$ of top predator (also see my response to Reviewer 2's comment 5).

Another potential limitation the reviewer pointed out is the nitrogen isotope enrichment from larvae to adults in phytophagous insects due metamorphosis metabolism. Consuming these ^{15}N-enriched prey items may lead to a high $\delta^{15}N$ 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 combined to form the overall IGP pattern (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 elaborate on 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 2 > Line 32: to study.

Response > Revised (Line 32).

Introduction

Comment 3 > 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" (also see my response to Reviewer 1's comment 2 on the citation format issue).

The proposed experimental framework

Comment 4 > 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:

"All experimental organisms are starved prior to the feeding trial to avoid potential contamination from their gut contents." (Line 101-103)

"(all experimental organisms are starved beforehand as in the first feeding trial)" (Line 107)

"These field-collected organisms are kept in starvation to empty their guts before preparation for stable isotope analysis." (Line 128-129)

Comment 5 > 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 the shared prey or mesopredator. I have discussed this in the sixth paragraph of the *Applications* section (Line 198-201):

"Furthermore, if top predator engages in cannibalism, multiple predator individuals (based on their field density) can be reared together in the same experimental arena to better reflect cannibalism events in the field and to account for their effects on the $\delta^{15}N$ of top predator."

Comment 6 > 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 N^{15} 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 107):

"(all experimental organisms are starved beforehand as in the first feeding trial)"

(Also see my response to Reviewer 2's comment 4 for more details on starving the experimental organisms.)

Comment 7 > 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 N^{15} 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 N^{15} 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 since it can feed on prey items other than the shared prey. I have discussed this issue in the fifth paragraph of the *Applications* section (Line 180-188):

"Mesopredator may consume prey items other than the shared prey in the field, thus leading to deviation of its $\delta^{15}N$ from that of the lab-reared mesopredator (which feeds entirely on the shared prey for constructing the standard IGP curve). To account for this potential source of variation in the $\delta^{15}N$ of mesopredator, researchers can analyze the nitrogen isotope signatures of mesopredator in the field and calibrate the $\delta^{15}N$ values of the field-collected top predator individuals by subtracting the average $\delta^{15}N$ difference between field-collected and lab-reared mesopredator individuals (such $\delta^{15}N$ difference is due to mesopredator feeding on alternative prey). The calibrated top predator $\delta^{15}N$ values can then be interpolated to the standard IGP curve to estimate the degree of IGP more accurately."

Applications

Comment 8 > Line 138: to study.

Response > Revised (Line 148).