**Title**

An experimental framework for quantifying the degree of intraguild predation in omnivorous food webs in the field

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**Running headline**

Framework quantifying intraguild predation

**Abstract**

Intraguild predation (IGP) is common in natural and human-managed systems and plays a critical role in food web dynamics. Although studies have documented the occurrence of IGP across a wide range of predator taxa, quantitative understanding regarding the degree/intensity of IGP remains lacking. I propose an experimental framework combining controlled feeding trials and stable isotope analysis to quantify the degree of IGP in a three-species omnivorous food web (top predator + mesopredator + shared prey) in the field. The degree of IGP is defined as the proportion (in number) of mesopredator consumed in the total diet (shared prey + mesopredator) of the top predator. Feeding trials along with stable isotope analysis are used to construct a standard curve of the relationship between the top predator’s diet and the shift in its nitrogen isotope signatures. The nitrogen isotope signatures of field-sampled top predator individuals are then analyzed and interpolated to the curve to estimate the degree of IGP in the field. The proposed framework leverages the strengths of different experimental approaches to study trophic interactions, providing a practical tool for quantifying IGP in a more accurate (controlled feeding trials and standard IGP curve) and realistic (stable isotope analysis of field samples) fashion. The current framework can be further extended to food webs involving more complex interactions (e.g., multiple shared prey) and complemented with other approaches (e.g., molecular gut content analysis) to capture a more complete picture of IGP dynamics in the field.

**Keywords**

feeding trial, food web, intraguild predation, omnivory, stable isotope analysis, trophic interactions

**Introduction**

Intraguild predation (IGP) is common in natural and human-managed ecosystems (Arim & Marquet 2004; Müller & Brodeur 2002; Polis & Holt 1992) and is documented across a wide range of predator taxa (Gagnon et al., 2011; Polis et al., 1989). IGP could substantially affect the abundance and distribution of interacting species, alter food web dynamics, and influence biodiversity and ecosystem functioning (Polis et al., 1989; Wang et al., 2019).

Previous studies have recorded the occurrence of IGP among arthropod predators through field observations of diet compositions (e.g., Birkhofer & Wolters 2012; Nyffeler & Sunderland 2003). Manipulative experiments in the field and laboratory have also been used to examine the intensity of IGP as a function of predator and prey density (e.g., Denno et al., 2004), allowing for causal inferences about the mechanisms underlying predator-predator interactions and its effect on prey population. Nonetheless, the confined settings in these experiments may potentially alter the encounter rates between organisms, thus leading to biased results (Uiterwaal et al., 2019).

Stable isotopes, particularly nitrogen isotope signatures (δ15N), have been used to estimate the trophic levels of predators for assessing IGP (e.g., Wise et al., 2006). It is suggested that IGP would increase the δ15N of predators (Ponsard & Arditi 2000). For example, Rickers et al., (2006) conducted feeding experiments on wolf spiders (*Alopecosa cuneata*) and found a higher δ15N of these top predators in IGP treatment. However, the study did not quantify the degree of IGP as the IGP treatment was binary (absence vs. presence of mesopredator) with constant numbers of shared prey and mesopredator. On top of that, the trophic levels of top predators in previous studies were often calculated based on assumed trophic discrimination factors (TDFs) (Svanbäck et al., 2015). Since TDFs are often taxon-specific (Caut et al., 2009), this could lead to incorrect trophic level estimates and inferences about IGP in the field.

Recently, researchers have applied molecular gut content analysis and immunological techniques to reliably detect the presence of certain food items in predators’ diet (Gagnon et al., 2011; Hagler et al., 2020), allowing for calculating the incidence rates of IGP (the probability of detecting mesopredator in top predator’s gut contents) (e.g., Snyder et al., 2022). Nonetheless, the incidence rates may not necessarily reflect the degree of IGP in the system (Raso et al., 2014). For example, it is possible that a high percentage of top predator individuals feed on mesopredator yet each of them consumes on average a low proportion of mesopredator in the diet. In this case, a high incidence rate of IGP only provides an incomplete picture of IGP dynamics. Studies have also quantified the strength of IGP based on the proportions of prey DNA reads (including mesopredator) in top predator’s gut contents (e.g., Saqib et al., 2021). This method provides useful quantitative information about IGP, yet the relative abundance of DNA sequences in gut contents is largely influenced by prey biomass and prey detectability in DNA extracts (Macías-Hernández et al., 2018), and therefore the proportion estimates might not reflect the relative *numbers* of prey consumed if the prey items differ substantially in their biomass or digestibility (Clare 2014).

Quantifying IGP is a critical step towards a deeper understanding of food web dynamics. Research has attempted to predict the intensity/degree of IGP based on allometric theory (Schneider et al., 2012), yet empirical evidence remains scarce. To address this gap, I propose an experimental framework combining controlled feeding trials and stable isotope analysis of field samples to estimate the degree of IGP in a three-species omnivorous food web (top predator + mesopredator + shared prey). The degree of IGP is defined herein as the proportion (in number) of mesopredator consumed in the total diet (mesopredator + shared prey) of top predator. The feeding trials will experimentally link different levels of mesopredator consumption by top predator to the changes in top predator’s nitrogen isotope signatures via an IGP standard curve, to which the nitrogen isotope signatures of field-collected top predator individuals are interpolated to estimate the degree of IGP in the field.

**The proposed experimental framework**

The proposed experimental framework consists of three main stages: (1) first feeding trial for stable isotope calibration of mesopredator and top predator, (2) second feeding trial for construction of standard IGP curve, and (3) collection of field samples for IGP estimation. I will illustrate the framework using an example of a terrestrial arthropod food web involving a spider top predator, a spider mesopredator, and a planthopper shared prey (Fig. 1a) in the following paragraphs.

The first feeding trial is to calibrate the nitrogen isotope signatures of the mesopredator and top predator. In this trial, the two predators are fed the shared prey for a period of time (Fig. 1b). All experimental organisms are starved prior to the feeding trial to avoid potential contamination from their gut contents. The actual duration of feeding may vary depending on the species. For arthropods, a period of 5–10 days will allow predators to incorporate isotope signatures into their tissues and reach an isotopic equilibrium state with the shared prey (Quinby et al., 2020).

The second feeding trial is to simulate a full range of omnivory that the top predator may exhibit in the field for constructing a standard IGP curve. In this trial, the top predator is fed different proportions of shared prey and mesopredator individuals from the first feeding trial: (1) 100% shared prey, (2) 75% shared prey + 25% mesopredator, (3) 50% shared prey + 50% mesopredator, (4) 25% shared prey + 75% mesopredator, and (5) 100% mesopredator (Fig. 1c). All experimental organisms are starved beforehand as in the first feeding trial. The actual numbers of shared prey and mesopredator supplied can be determined based on their feeding rates, obtained through either field observations or literature. To avoid the potential interfering effects of mesopredator feeding on the shared prey, the prey items are presented to the top predator one at a time in a randomized sequence instead of all at once. This also allows the researcher to ensure that a prey item is consumed by the top predator before the next item is presented.

At the end of the second trial (which has same duration as the first feeding trial to allow for the incorporation of prey isotope signatures into predator’s tissues), the top predator individuals in each diet treatment as well as the shared prey are prepared for stable isotope analysis to obtain their δ15N values. The difference in δ15N between the top predator and the shared prey is computed (δ15N*predator*─ δ15N*prey*; experimental Δ15N), and a standard curve is constructed by fitting a non-linear regression on the experimental Δ15N against the proportion of mesopredator in the diet (Fig. 1d).

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; 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). If possible, these field-collected organisms should be kept in starvation to empty their guts before preparation for stable isotope analysis. The shared prey individuals are pooled to obtain an average shared prey δ15N, while the nitrogen isotope signatures of top predator individuals are analyzed separately so that each individual has its own δ15N. The degree of IGP for each top predator individual is then estimated by interpolating its empirical Δ15N (calculated as individual top predator δ15N ─ average shared prey δ15N) to the standard IGP curve (Fig. 1e). The mean and standard error of individual IGP estimates can provide a measure of the average degree of IGP in the field and the uncertainty around the mean estimate at the population level.

A hypothetical example of standard IGP curve construction and estimation of IGP with field samples is shown in Fig. 2. In this example, five diet treatments are used; each treatment contains five top predator individuals, each of which is fed 12 prey items during the feeding period. After the feeding trial, the experimental Δ15N of these predator individuals are determined and used to construct a standard IGP curve (Fig. 2a). 20 top predator and 30 shared prey individuals are then collected from the field for stable isotope analysis and determination of empirical Δ15N. An IGP estimate is calculated for each top predator individual and therefore there will be a total of 20 estimates, which are further averaged to quantify the degree of IGP at the population level (Fig. 2b).

**Applications**

The proposed framework leverages the strengths of different approaches to study trophic interactions—the controlled feeding trials combined with stable isotope analysis can yield accurate experimental Δ15N for constructing a standard IGP curve, whereas the empirical Δ15N derived from field samples reflects the trophic interactions under natural settings. Additionally, the framework is robust to variations in background isotope signatures because the IGP estimation is based on the difference in nitrogen isotope signatures (Δ15N) rather than the original values (δ15N), thus allowing for comparisons across sites or systems with distinct background isotope signatures.

The framework can be implemented along environmental gradients or under different field treatments to investigate how various abiotic and biotic factors affect IGP interactions of a certain food web type (e.g., arthropod food web). For instance, one can quantify and compare the degree of IGP across altitudes to examine whether omnivory patterns change with temperature, precipitation, or vegetation (e.g., Michalko et al., 2022). Moreover, this study gives an example of arthropod food web with spider as the top predator, but the framework applies to other generalist predators as well, provided that they are amenable to feeding trials and available for collection in the field.

Systems with clear IGP patterns and relatively simple trophic interaction networks are ideal for implementing the proposed framework, as this can minimize the interference of non-focal species on the IGP interactions among focal organisms (Vance-Chalcraft et al., 2007). One of such systems is agricultural system, in which IGP occurs frequently (Polis et al., 1989; Rosenheim et al., 1995) and the food webs are generally less complex compared to most natural ecosystems. Furthermore, understanding the degree of IGP in agricultural field can provide useful implications for practitioners, for example, evaluating the effectiveness of biocontrol agents in pest control programs (Müller & Brodeur 2002).

The nitrogen isotope signatures of organisms can change over developmental stages. For example, holometabolous insects such as Lepidoptera and Diptera species show 15N enrichment from larvae to adults as a result of protein metabolism during metamorphosis (Tibbets et al., 2008). Feeding on such 15N-enriched prey could lead to a high δ15N of top predator that is not due to IGP. In this case, researchers can apply the proposed framework using prey individuals at different developmental stages in separate feeding trials to derive stage-specific standard IGP curves (the curve can indeed be a decreasing function of the degree of IGP if the δ15N of the shared prey is higher than that of the mesopredator). The stage-specific IGP estimates can then be linked together to form the overall IGP pattern.

Mesopredator may consume prey items other than the shared prey in the field, thus leading to deviation of its δ15N 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, researchers can analyze the nitrogen isotope signatures of mesopredator in the field and calibrate the δ15N values of the field-collected top predator individuals by subtracting the average δ15N difference between field-collected and lab-reared mesopredator individuals (i.e., such δ15N difference is due to mesopredator feeding on alternative prey). The calibrated top predator δ15N values can then be used to more accurately estimate the degree of IGP.

A potential limitation of the proposed framework is that the top predator individuals can feed on multiple prey items in the field. Researchers can conduct field observations or molecular gut content analysis to identify the non-focal prey consumed in top predator’s diet, collect field samples of non-focal prey and analyze their nitrogen isotope signatures, and adjust the δ15N of top predator for IGP estimation. For instance, suppose that the top predator consumes 15% of non-focal prey that has an average δ15N value 1.0‰ higher than that of the focal shared prey, then one can subtract 0.15‰ (15% × 1.0‰) from the δ15N of top predator individuals assuming linear stable isotope mixing (Boecklen et al., 2011). Alternatively, researchers can directly include non-focal prey items in the feeding trials to account for their effects on intraguild interactions and thus the δ15N of top predator. 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 δ15N of top predator. Lastly, collecting sufficiently large field samples of top predator will yield an average IGP estimate that fairly captures the overall IGP pattern at the population level.

Recent advance in compound-specific isotope analysis of amino acids (CSIA-AA)

One of the major advantages of CSIA-AA is that it can be used to estimate trophic positions (TP) of consumers even when some of their prey items are unknown to researchers. A potential way in which CSIA-AA can aid in the proposed framework is to compare the TP of field-collected and lab-reared top predator. Theoretically, if top predator consumes more non-focal prey items in the field, then its TP will be more different from (and presumably much lower than) that of the lab-reared top predator. Thus, the amount of deviation from TP of lab-reared top predator can allow researchers to gauge the actual degree of IGP in the field relative to the one estimated via controlled feeding trials. Additional measures can be taken to account for the effects of non-focal prey on IGP interactions.

A better quantitative understanding of IGP can offer critical insights into the complex predator-predator-prey trophic interactions and help predict the community structure and stability (Arim & Marquet 2004; Pahl et al., 2020). I am optimistic about the practical applications of the proposed framework and future experiments to validate and refine it. The current framework can be extended to food webs involving more complex interactions (e.g., multiple shared prey) and further complemented with other approaches (e.g., combining the degree of IGP at the population level with the incidence rates derived from molecular gut content analysis to estimate the total IGP impact) to better elucidate the IGP dynamics in the field.

**Acknowledgements**

I thank Jia-Ang (William) Ou and Po-Ju Ke for the useful comments on this manuscript.

**Conflict of interest**

The author declares no conflict of interest regarding this manuscript.

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