INTERDISCIPLINARY PERSPECTIVE

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

G.-C. Hsu

Department of Life Science, National Taiwan University, Taipei, Taiwan

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 an omnivorous food web in the field consisting of a top predator, a mesopredator, and a shared prey. The degree of IGP is defined as the proportion (in number) of mesopredators consumed in the total diet (shared prey + mesopredator) of top predators. Feeding trials along with stable isotope analysis are used to construct a standard curve of the relationship between the diet composition of top predator and its nitrogen isotope signatures. The nitrogen isotope signatures of field-collected top predator individuals are then analyzed and interpolated to the standard 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.

Introduction

Intraguild predation (IGP) is common in natural and human-managed ecosystems (Arim & Marquet, 2004; Müller & Brodeur, 2002; Polis & Holt, 1992) and has been 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).

Studies have recorded the occurrence of IGP among arthropod predators through field observations of diet compositions (Birkhofer & Wolters, 2012; Nyffeler & Sunderland, 2003). Manipulative experiments under both field and laboratory settings have also been conducted to examine the intensity of IGP as a function of predator and prey density (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 could potentially alter the encounter rates between organisms and thus lead to biased results (Uiterwaal et al., 2019).

Nitrogen stable isotope (^{15}N) has been used to estimate the trophic levels of predators for assessing IGP (Wise et al., 2006). It is suggested that IGP would increase the nitrogen isotope ratio (δ^{15}N) of predators (Ponsard & Arditi, 2000). For example, Rickers et al. (2006) conducted feeding experiments on wolf spider (*Alopecosa cuneata*) and found a higher δ^{15}N in IGP treatment. However, the study was not able to quantify the degree of IGP because the IGP treatment was binary (absence vs. presence of mesopredator) with constant numbers of shared prey and mesopredator. Moreover, the trophic levels of top predators in previous studies were often calculated based on the 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.

Molecular gut content analysis and immunological techniques can reliably detect the presence of certain food items in consumer's diet (Gagnon et al., 2011; Hagler et al., 2020), allowing researchers to calculate the incidence rates of IGP (the probability of detecting mesopredator in top predator's gut contents) (Snyder et al., 2022). Nonetheless, incidence rates

may not capture the full picture of IGP in the system (Raso et al., 2014). For instance, it is possible that a high percentage of top predator individuals feed on mesopredator and hence a high incidence rate, but each of them consumes only a low proportion of mesopredator in the diet. Studies have also quantified the strength of IGP based on the proportions of prey DNA reads (including mesopredator) in top predator's gut contents (Saqib et al., 2021). This method provides useful quantitative information about IGP, yet the relative abundance of DNA sequences in gut contents can be largely influenced by prey biomass and prey detectability in DNA extracts (Macías-Hernández et al., 2018). If prey items differ substantially in their biomass or digestibility, then the proportions of prey DNA in predator's gut contents will not reflect the relative *numbers* of prey consumed (Clare, 2014).

Quantifying IGP is a critical step toward 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), but 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 an omnivorous food web consisting of a top predator, a mesopredator, and a shared prey. The degree of IGP is defined as the proportion (in number) of mesopredator consumed in the total diet (mesopredator + shared prey) of top predator. The controlled feeding trials experimentally link different levels of mesopredator consumption by top predator to the changes in top predator's nitrogen isotope signatures via a standard IGP 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 in the following paragraphs using an example of a terrestrial arthropod food web involving a spider top predator, a spider mesopredator, and a planthopper shared prey (Fig. 1a).

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

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 (all experimental organisms are starved

beforehand as in 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). To avoid the potential interfering effect 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 allows the researcher to ensure that a prey item is consumed by the top predator before the next item is presented. The actual numbers of shared prey and mesopredator supplied are determined based on their feeding rates, obtained through either field observations or literature.

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), top predator individuals in each diet treatment as well as the shared prey are prepared for stable isotope analysis to obtain their $\delta^{15}N$ values. The difference in $\delta^{15}N$ between top predator and the shared prey is computed ($\delta^{15}N_{predator} - \delta^{15}N_{prey}$; experimental $\Delta^{15}N$), and a standard curve is constructed by fitting a non-linear regression on the experimental $\Delta^{15}N$ against the proportion of mesopredator consumed in the diet (Fig. 1d).

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). These field-collected organisms are 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 $\delta^{15}N$, whereas top predator individuals are analyzed separately so that each individual has its own $\delta^{15}N$. The degree of IGP for each top predator individual is then estimated by interpolating its empirical $\Delta^{15}N$ (calculated as individual top predator $\delta^{15}N$ – average shared prey $\delta^{15}N$) 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 at the population level and the uncertainty around the mean estimate.

A hypothetical example of standard IGP curve construction and IGP estimation 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 $\Delta^{15}N$ values of top predator individuals are derived to construct a standard IGP curve (Fig. 2a). Next, 20 top predator and 30 shared prey individuals are collected from the field for stable isotope analysis to obtain empirical $\Delta^{15}N$ value for each top predator individual. Last, the empirical $\Delta^{15}N$ values are interpolated to the standard IGP curve to estimate the degree of IGP at the individual level, and these individual-level IGP estimates 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 IGP interactions—the controlled feeding
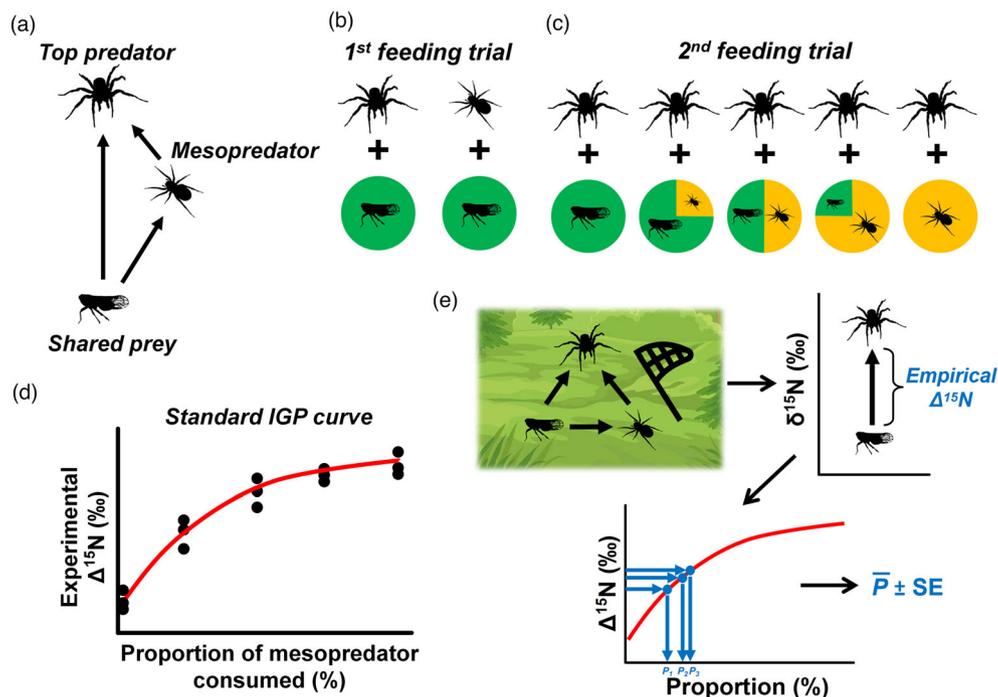
Figure 1 A schematic diagram of the proposed experimental framework for quantifying the degree of intraguild predation (IGP) in an omnivorous food web consisting of a spider top predator, a spider mesopredator, and a planthopper shared prey. See "The proposed experimental framework" section for detailed descriptions of the framework procedures.

trials combined with stable isotope analysis can yield accurate experimental $\Delta^{15}N$ for constructing a standard IGP curve, whereas the empirical $\Delta^{15}N$ values derived from field samples reflect 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 ($\Delta^{15}N$) rather than the raw values ($\delta^{15}N$), 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 in a given food web type (e.g., arthropod food web). For instance, researchers can quantify and compare the degree of IGP across altitudes to examine whether omnivory patterns change with temperature, precipitation, or vegetation (Michalko et al., 2022). Moreover, besides the given spider top predator example, 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 suited for implementing the proposed framework, which can minimize the interference of non-focal species on IGP interactions among focal organisms (Vance-Chalcraft et al., 2007). One of such systems is agricultural systems, 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. Some holometabolous insects such as Lepidoptera and Diptera species exhibit significant ^{15}N enrichment from larvae to adults as a result of protein metabolism during metamorphosis (Tibbets et al., 2008). Feeding on such ^{15}N-enriched prey could lead to a high $\delta^{15}N$ 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 construct stage-specific standard IGP curves (the curve can even be a decreasing function of the degree of IGP if the $\delta^{15}N$ of the shared prey is higher than that of the mesopredator). The stage-specific IGP estimates can then be combined 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 $\delta^{15}N$ 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
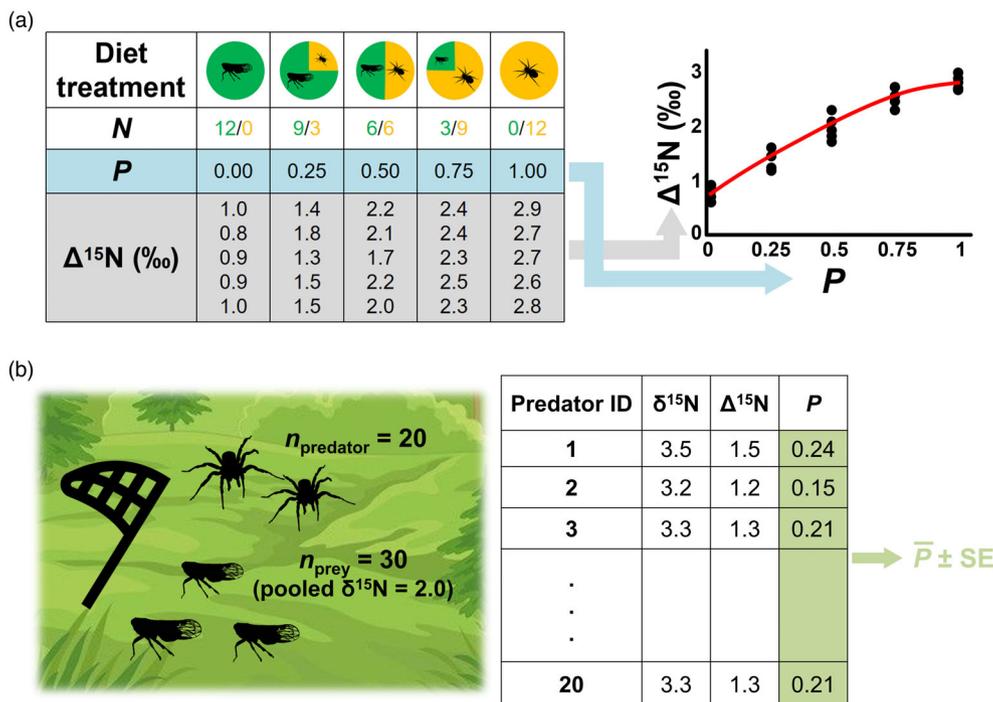
Figure 2 A hypothetical example of (a) standard IGP curve construction in the second feeding trial and (b) IGP estimation using field samples of top predator and the shared prey. N: number of shared prey/mesopredator in the mixed diet; P: proportion of mesopredator consumed by top predator (%).

standard IGP curve to estimate the degree of IGP more accurately.

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 $\delta^{15}N$ of top predator for IGP estimation. For instance, suppose that the top predator consumes 15% of non-focal prey that has an average $\delta^{15}N$ value 1.0‰ higher than that of the focal shared prey, then researchers can subtract 0.15‰ (15% × 1.0‰) from the $\delta^{15}N$ of top predator individuals assuming linear stable isotope mixing (Boecklen et al., 2011). Alternatively, researchers can directly include non-focal prey in the feeding trials to account for their effects on IGP interactions and thus the $\delta^{15}N$ 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 $\delta^{15}N$ of top predator. Last, collecting sufficiently large field samples of top predator will yield an average IGP estimate that better captures the IGP pattern at the population level.

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).

A better quantitative understanding of IGP can shed light on 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 refinement through experiments. 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., combining the individual-level IGP estimates with the incidence rates derived from molecular gut content

analysis to evaluate the total IGP effect) to better elucidate the IGP dynamics in the field.

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Conflict of interest

The author declares no conflict of interest regarding this manuscript.

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