1 **Title**

2 An experimental framework for quantifying the degree of intraguild predation in omnivorous

3 food webs in the field

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16 **Running headline**

17 Framework quantifying intraguild predation

18

19 **Abstract**

20 Intraguild predation (IGP) is common in natural and human-managed systems and plays a

21 critical role in food web dynamics. Although studies have documented the occurrence of IGP

22 across a wide range of predator taxa, quantitative understanding regarding the degree/intensity

23 of IGP remains lacking. I propose an experimental framework combining controlled feeding

24 trials and stable isotope analysis to quantify the degree of IGP in an omnivorous food web in

25 the field consisting of top predator, mesopredator, and shared prey. The degree of IGP is

26 defined as the proportion (in number) of mesopredator consumed in the total diet (shared prey

27 + mesopredator) of top predator. Feeding trials along with stable isotope analysis are used to

28 construct a standard curve of the relationship between the diet composition of top predator and

29 its nitrogen isotope signatures. The nitrogen isotope signatures of field-collected top predator

30 individuals are then analyzed and interpolated to the standard curve to estimate the degree of

31 IGP in the field. The proposed framework leverages the strengths of different experimental

32 approaches to study trophic interactions, providing a practical tool for quantifying IGP in a

33 more accurate (controlled feeding trials and standard IGP curve) and realistic (stable isotope

34 analysis of field samples) fashion. The current framework can be further extended to food webs

35 involving more complex interactions (e.g., multiple shared prey) and complemented with other

36 approaches (e.g., molecular gut content analysis) to capture a more complete picture of IGP

37 dynamics in the field.

38

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 (e.g., 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 (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 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 (e.g., 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.

66 Molecular gut content analysis and immunological techniques can reliably detect the

67 presence of certain food items in consumer's diet (Gagnon et al., 2011; Hagler et al., 2020),

68 allowing researchers to calculate the incidence rates of IGP (the probability of detecting

69 mesopredator in top predator's gut contents) (e.g., Snyder et al., 2022). Nonetheless, incidence

70 rates may not capture the full picture of IGP in the system (Raso et al., 2014). For instance, it

71 is possible that a high percentage of top predator individuals feed on mesopredator and hence

72 a high incidence rate, but each of them consumes only a low proportion of mesopredator in the

73 diet. Studies have also quantified the strength of IGP based on the proportions of prey DNA

74 reads (including mesopredator) in top predator's gut contents (e.g., Saqib et al., 2021). This

75 method provides useful quantitative information about IGP, yet the relative abundance of DNA

76 sequences in gut contents can be largely influenced by prey biomass and prey detectability in

77 DNA extracts (Macías-Hernández et al., 2018). If prey items differ substantially in their

78 biomass or digestibility, then the proportions of prey DNA in predator's gut contents will not

79 reflect the relative *numbers* of prey consumed (Clare 2014).

80 Quantifying IGP is a critical step towards a deeper understanding of food web dynamics.

81 Research has attempted to predict the intensity/degree of IGP based on allometric theory

82 (Schneider et al., 2012), but empirical evidence remains scarce. To address this gap, I propose

83 an experimental framework combining controlled feeding trials and stable isotope analysis of

84 field samples to estimate the degree of IGP in an omnivorous food web consisting of top

85 predator, mesopredator, and the shared prey. The degree of IGP is defined as the proportion (in

86 number) of mesopredator consumed in the total diet (mesopredator + shared prey) of top

87 predator. The controlled feeding trials experimentally link different levels of mesopredator

88 consumption by top predator to the changes in top predator's nitrogen isotope signatures via a

89 standard IGP curve, to which the nitrogen isotope signatures of field-collected top predator

90 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

115   are determined based on their feeding rates, obtained through either field observations or

116   literature.

117   At the end of the second trial (which has same duration as the first feeding trial to allow

118   for the incorporation of prey isotope signatures into predator's tissues), top predator individuals

119   in each diet treatment as well as the shared prey are prepared for stable isotope analysis to

120   obtain their $\delta^{15}N$ values. The difference in $\delta^{15}N$ between top predator and the shared prey is

121   computed ($\delta^{15}N_{predator} - \delta^{15}N_{prey}$; experimental $\Delta^{15}N$), and a standard curve is constructed by

122   fitting a non-linear regression on the experimental $\Delta^{15}N$ against the proportion of mesopredator

123   consumed in the diet (Fig. 1d).

124   Finally, field samples of the top predator and shared prey are collected under

125   homogeneous site conditions (e.g., similar ambient temperature and vegetation structure) to

126   minimize the potential confounding effects of abiotic and biotic factors. The spatial scale at

127   which the samples are taken should pertain to the research goal (e.g., various locations within

128   a farm to quantify farm-level IGP, or various plots within a one-hundred-hectare grassland to

129   quantify community-level IGP). These field-collected organisms are kept in starvation to

130   empty their guts before preparation for stable isotope analysis. The shared prey individuals are

131   pooled to obtain an average shared prey $\delta^{15}N$, whereas top predator individuals are analyzed

132   separately so that each individual has its own $\delta^{15}N$. The degree of IGP for each top predator

133   individual is then estimated by interpolating its empirical $\Delta^{15}N$ (calculated as individual top

134   predator $\delta^{15}N$ — average shared prey $\delta^{15}N$) to the standard IGP curve (Fig. 1e). The mean and

135   standard error of individual IGP estimates can provide a measure of the average degree of IGP

136   in the field at the population level and the uncertainty around the mean estimate.

137   A hypothetical example of standard IGP curve construction and IGP estimation is

138   shown in Fig. 2. In this example, five diet treatments are used; each treatment contains five top

139 predator individuals, each of which is fed 12 prey items during the feeding period. After the

140 feeding trial, the experimental $\Delta^{15}N$ values of top predator individuals are derived to construct

141 a standard IGP curve (Fig. 2a). Next, 20 top predator and 30 shared prey individuals are

142 collected from the field for stable isotope analysis to obtain empirical $\Delta^{15}N$ value for each top

143 predator individual. Lastly, the empirical $\Delta^{15}N$ values are interpolated to the standard IGP

144 curve to estimate the degree of IGP at the individual level, and these individual-level IGP

145 estimates are further averaged to quantify the degree of IGP at the population level (Fig. 2b).

146

147 **Applications**

148   The proposed framework leverages the strengths of different approaches to study IGP

149 interactions—the controlled feeding trials combined with stable isotope analysis can yield

150 accurate experimental $\Delta^{15}N$ for constructing a standard IGP curve, whereas the empirical $\Delta^{15}N$

151 values derived from field samples reflect trophic interactions under natural settings.

152 Additionally, the framework is robust to variations in background isotope signatures because

153 the IGP estimation is based on the difference in nitrogen isotope signatures ($\Delta^{15}N$) rather than

154 the raw values ($\delta^{15}N$), thus allowing for comparisons across sites or systems with distinct

155 background isotope signatures.

156   The framework can be implemented along environmental gradients or under different

157 field treatments to investigate how various abiotic and biotic factors affect IGP interactions in

158 a given food web type (e.g., arthropod food web). For instance, researchers can quantify and

159 compare the degree of IGP across altitudes to examine whether omnivory patterns change with

160 temperature, precipitation, or vegetation (e.g., Michalko et al., 2022). Moreover, besides the

161 given spider top predator example, the framework applies to other generalist predators as well,

162 provided that they are amenable to feeding trials and available for collection in the field.

163   Systems with clear IGP patterns and relatively simple trophic interaction networks are

164   suited for implementing the proposed framework, which can minimize the interference of non-

165   focal species on IGP interactions among focal organisms (Vance-Chalcraft et al., 2007). One

166   of such systems is agricultural systems, in which IGP occurs frequently (Polis et al., 1989;

167   Rosenheim et al., 1995) and the food webs are generally less complex compared to most natural

168   ecosystems. Furthermore, understanding the degree of IGP in agricultural field can provide

169   useful implications for practitioners, for example, evaluating the effectiveness of biocontrol

170   agents in pest control programs (Müller & Brodeur 2002).

171   The nitrogen isotope signatures of organisms can change over developmental stages.

172   Some holometabolous insects such as Lepidoptera and Diptera species exhibit significant ^{15}N

173   enrichment from larvae to adults as a result of protein metabolism during metamorphosis

174   (Tibbets et al., 2008). Feeding on such ^{15}N-enriched prey could lead to a high δ^{15}N of top

175   predator that is not due to IGP. In this case, researchers can apply the proposed framework

176   using prey individuals at different developmental stages in separate feeding trials to construct

177   stage-specific standard IGP curves (the curve can even be a decreasing function of the degree

178   of IGP if the δ^{15}N of the shared prey is higher than that of the mesopredator). The stage-specific

179   IGP estimates can then be combined to form the overall IGP pattern.

180   Mesopredator may consume prey items other than the shared prey in the field, thus

181   leading to deviation of its δ^{15}N from that of the lab-reared mesopredator (which feeds entirely

182   on the shared prey for constructing the standard IGP curve). To account for this potential source

183   of variation in the δ^{15}N of mesopredator, researchers can analyze the nitrogen isotope

184   signatures of mesopredator in the field and calibrate the δ^{15}N values of the field-collected top

185   predator individuals by subtracting the average δ^{15}N difference between field-collected and

186   lab-reared mesopredator individuals (such δ^{15}N difference is due to mesopredator feeding on

187   alternative prey). The calibrated top predator $\delta^{15}N$ values can then be interpolated to the

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

189   A potential limitation of the proposed framework is that the top predator individuals

190   can feed on multiple prey items in the field. Researchers can conduct field observations or

191   molecular gut content analysis to identify the non-focal prey consumed in top predator's diet,

192   collect field samples of non-focal prey and analyze their nitrogen isotope signatures, and adjust

193   the $\delta^{15}N$ of top predator for IGP estimation. For instance, suppose that the top predator

194   consumes 15% of non-focal prey that has an average $\delta^{15}N$ value 1.0‰ higher than that of the

195   focal shared prey, then researchers can subtract 0.15‰ (15% × 1.0‰) from the $\delta^{15}N$ of top

196   predator individuals assuming linear stable isotope mixing (Boecklen et al., 2011).

197   Alternatively, researchers can directly include non-focal prey in the feeding trials to account

198   for their effects on IGP interactions and thus the $\delta^{15}N$ of top predator. Furthermore, if top

199   predator engages in cannibalism, multiple predator individuals (based on their field density)

200   can be reared together in the same experimental arena to better reflect cannibalism events in

201   the field and to account for their effects on the $\delta^{15}N$ of top predator. Lastly, collecting

202   sufficiently large field samples of top predator will yield an average IGP estimate that better

203   captures the IGP pattern at the population level.

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

205   provide a promising tool for studying trophic interactions (see Ishikawa [2018] and McMahon

206   & McCarthy [2016] for more details on the use of CSIA-AA in trophic ecology). A major

207   advantage of CSIA-AA over bulk stable isotope analysis is that it can estimate trophic positions

208   (TP) of consumers even when some of their prey items are unknown to researchers. A potential

209   application of CSIA-AA that may aid in the proposed framework is to analyze and compare

210   the TP of field-collected and lab-reared top predator. Theoretically, if top predator consumes

211   more non-focal prey items in the field, its TP will deviate more from (presumably be lower

212   than) that of the lab-reared top predator because it incorporates more lower-level biomass into

213   the tissue. In this regard, the amount of deviation can allow researchers to gauge the actual

214   degree of IGP in the field relative to the one estimated via the controlled feeding trial (e.g., a

215   larger deviation in TP may indicate a lower actual degree of IGP in the field compared to the

216   IGP estimated via the feeding trial). Additional measures can be taken to account for the effects

217   of non-focal prey on IGP interactions (see previous paragraph for details).

218   A better quantitative understanding of IGP can shed light on the complex predator-

219   predator-prey trophic interactions and help predict the community structure and stability (Arim

220   & Marquet 2004; Pahl et al., 2020). I am optimistic about the practical applications of the

221   proposed framework and future refinement through experiments. The current framework can

222   be further extended to food webs involving more complex interactions (e.g., multiple shared

223   prey) and complemented with other approaches (e.g., combining the individual-level IGP

224   estimates with the incidence rates derived from molecular gut content analysis to evaluate the

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

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229

230   **Conflict of interest**

231   The author declares no conflict of interest regarding this manuscript.

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