**Running title**

Stable isotope analysis reveals consistent pest consumption by generalist arthropod predators (ladybeetles and spiders) in rice farms

* A predator in need is a predator indeed
* Generalist predators are specialists when things matter (during high pest density)

**Abstract**

**Introduction**

* [Importance of biocontrol]

1. Importance of biocontrol service in agriculture (e.g., using predators to control pests): a long history in agriculture (e.g., buy and place ant nests in orchards in China); critical as well in Anthropocene (e.g., 50% pesticide reduction in EU by 2030)

* [Importance of GAPs]

1. Previous studies on the potential of (underappreciated) generalist arthropod predators (GAPs) as biocontrol agents: cages, observations, molecular gut content analysis
2. Strengths and limitations of previous approaches (e.g., MGCA only provides qualitative information on predators’ diet compositions [presence/absence of prey in predators’ diet])

* [Knowledge gaps]

1. The diet composition of GAPs is a key determinant of the top-down control, reflecting their foraging habits and the trophic interactions with their prey. To fully realize the potential of GAPs, we need to quantify their diet compositions during crop growing season (crop stages). Moreover, to understand the reliability of GAPs as biocontrol agents over environmental fluctuations, it is also important to examine the consistency of pest consumption by GAPs across years in the field.
2. A better understanding of how various biotic and abiotic factors may affect the diet compositions of GAPs is crucial for predicting whether GAPs could provide reliable and stable biological control services in agro-ecosystems as well as provide insights (mechanisms) for a better agricultural management

* [Aims]

1. Quantify diet compositions of GAPs (predator guild, spider and ladybeetle group) over crop stages
2. Examine the consistency of rice herbivore composition by GAPs(rice herbivores) over time (years)
3. Investigate how biotic and abiotic factors may affect the diet compositions of GAPs.

* Brief introduction of our system: stable isotope approach and feeding guilds

[Broad overview of biocontrol in the past and in the modern days]

Using natural arthropod enemies for pest control has a long history in agriculture. For example, the earliest record of biocontrol was documented in the book *Plants of the Southern Regions* (ca. 304 A.D.) that people in Southern China sold ants and their nests (attached to branches) in market to control citrus insect pests (Huang and Yang 1987). With the advent of new technologies in the past century, synthetic pesticides have become the main method to control pests in agriculture. However, this comes at a cost, such as posing risks to people, reducing biodiversity (e.g., decline in top predators) and hampering ecosystem functions (e.g., decline in pollinator service) (Geiger et al. 2010, Kehoe et al. 2017). As agriculture has become the largest land use type worldwide and the major driver for global biodiversity crisis and environmental degradation in Anthropocene (Campbell et al. 2017), a shift from synthetic pesticides to environmental-friendly practices (e.g., biocontrol) is urgently needed to make agriculture more sustainable (Gomiero et al. 2011). For example, the European Commission has recently announced its plan to reduce the use chemical pesticides in European Union’s agricultural systems by 50% by 2030 (EC 2020). Such large-scale plan for pesticide reduction will open great opportunities for pest control by natural enemies. In light of this, biocontrol by enemies to reduce pesticide applications has regained its importance nowadays and become an area of increasing focus in sustainable agriculture.

[Brief introduction of specialists vs. generalist arthropod enemies]

The natural arthropod enemies for herbivorous insect pest control can be classified into two major groups based on their host/prey range—specialists and generalist predators. On one hand, specialist enemies (e.g., parasitoids) have been widely applied in agriculture because they are able to target specific pest species and thus minimizes the undesirable non-target effects (Stiling and Cornelissen 2005). On the other hand, generalist predators (e.g., spiders) could feed on not only target pests but also non-pest alternative prey due to their polyphagous nature. Consequently, their effectiveness as biocontrol agents has long been questioned (Symondson et al. 2002), and whether generalist predators can provide reliable top-down control on crop pests remains a subject of much debate.

[Review of previous studies on biocontrol by generalist predators]

Empirical evidence for the biocontrol effectiveness of generalist predators remains mixed and may be context-dependent. Some studies have suggested that generalist predators can be effective in controlling pest populations. For example, a review shows that generalist predators significantly decreased pest abundances in around 75% cases of the 181 field manipulative studies examined (Symondson et al. 2002). Moreover, a meta-analysis reveals that generalist predators exert stronger suppressing effects on pest populations compared with specialists (Stiling and Cornelissen 2005). On the other hand, it has also been shown that generalist predators can exhibit prey switching in the presence of alternative prey, which distracts predators from attacking target pests and thus weakens pest control (Michalko et al. 2019).

[Current knowledge gaps in biocontrol by generalist predators]  
 A few limitations exist in previous studies on pest control by generalist predators in agro-ecosystems. First, recent studies have applied molecular gut content analysis to determine the trophic linkages between predators and their prey (Eitzinger and Traugott 2011, Ingrao et al. 2017, Albertini et al. 2018). However, this approach provides only qualitative information on predator’s diet but does not actually quantify their diet compositions. Moreover, many studies examining the effects of predators on pests were conducted with cage experiments (Birkhofer et al. 2008b, Crowder et al. 2010, Muñoz-Cárdenas et al. 2017). Although this approach can provide direct causal effects of predators on pests, it might not account for the natural predator and prey population fluctuations, which could largely influence predator-prey interactions and thus the biocontrol by predators. Furthermore, such confined settings may potentially increase the encounter rates between predators and pests (Sih et al. 1985), thus leading to biased biocontrol results. Therefore, more studies conducted under natural settings are needed to get a more realistic picture of pest consumption by predators in agro-ecosystems.

Rearrange our sentences in the next three paragraphs > Paragraph 1 for aim 1 (quantify diet composition, over growth season), 2 for 2 (consistency over years; why important if lack of studies [predictability in management]), 3 for 3 (examine abiotic and biotic factors driving diet composition).

[Aim 1 and 2: quantify the diet compositions of predators over the crop season and examine the consistency in pest consumption across years]

To fully realize the biocontrol potential of generalist predators, it is important to first understand their diet compositions in the field. The diet compositions of predators directly reflect their trophic interactions with prey and are key determinants of their top-down control on pests. Therefore, quantifying the diet compositions of predators in the farms over the course of the crop season is the critical step toward untangling the complex predator-prey trophic dynamics. Moreover, to assess the reliability of predators as biocontrol agents under temporal environmental fluctuations, it is also important to examine their consistency in pest consumption over years.

[Aim 3: How various abiotic and biotic factors affect the diet compositions of predators]

Various factors can affect the diet compositions of generalist predators in agro-ecosystems. First, arthropod communities may vary over the season through crop development, and such temporal variations in arthropod compositions can affect predator-prey trophic interactions and thus the diet compositions of predators (Roubinet et al. 2017). Second, farming practices (e.g., organic and conventional farming) can influence arthropod community compositions and densities in the field, which may in turn alter the diet compositions of predators (Birkhofer et al. 2011). For instance, organic farming generally promotes arthropod prey diversity in the farms (Bengtsson et al. 2005), and higher prey diversity may increase predators’ diet breadths as a result of greater prey availability. In contrast, the application of synthetic chemicals can reduce the abundances of non-pest alternative prey (e.g., detritivores) but increase those of certain pest herbivores (Birkhofer et al. 2008a), potentially leading to higher pest consumption in predators’ diet. Third, the relative abundances of different prey items in the field could largely determine predators’ diet if predators forage in a prey-density-dependent fashion. Yet, some predators may exhibit prey preferences and their diet compositions may not directly reflect prey availability (Kuusk and Ekbom 2012, Eitzinger et al. 2019). For example, a study found that wolf spiders fed continually on pest species even under increasing densities of alternative prey (Wise et al. 2006). Finally, surrounding vegetation could affect predators’ diet by influencing the local arthropod species pool as well as the spatial distributions of predator and prey individuals. Vegetation complexity has been shown to affect prey capture and thus the diet compositions of web-building spiders (Diehl et al. 2013); greater habitat heterogeneity increases the diet breadths of predators as a result of relaxed intraspecific competition (Staudacher et al. 2018). As prey capture and diet compositions of generalist predators are the key determinants of their biocontrol effectiveness, understanding how these aforementioned factors may affect prey consumption by predators can provide useful information for designing agricultural management schemes that enhance their biocontrol services.

[Aims, strengths of our method, brief description of the study system and the key findings]

In this study, we used stable isotope analysis to (1) quantify the diet compositions of generalist arthropod predators (ladybeetles and spiders) in rice agro-ecosystems over three consecutive years, (2) examine the annual patterns of pest consumption by predators (i.e., the proportion of rice pests consumed in predators’ diet), and (3) investigate how various local factors (farm type, crop stage, percent forest cover, and the relative abundance of pests in the field) may affect pest consumption by predators. Stable isotope analysis has been widely applied in ecological studies to infer predator-prey trophic interactions and to estimate the proportions of different prey sources in predators’ diet (Post 2002, Boecklen et al. 2011, Layman et al. 2012). This approach has advantages over other “snap-shot” techniques (e.g., field observations and gut content analysis) in that it can provide time-integrated dietary information of predators (Newton 2016). We sampled terrestrial arthropods in subtropical organic and conventional rice farms throughout the course of the crop season over three consecutive years (2017-2019). We found that predators consumed on average high proportion of rice pests in the diet, and the proportion increased over crop stages. Moreover, such pest consumption patterns were similar across the three study years. Interestingly, predators in conventional farms consumed higher proportion of rice pests in the diet compared with predators in organic farms. Our results could provide a deeper insight into the potential of generalist arthropod predators as biocontrol agents in agro-ecosystems.

**Materials and Methods**

*Study system and sample collection*

We collected terrestrial arthropods in organic and conventional rice farms in Miaoli County, Taiwan, from 2017 to 2019 (three farms each in 2017 and seven farms each in 2018 and 2019). [These farms were typical subtropical rice farms, with a mean area of 0.2 hectares and irrigated with surface water. Organic fertilizers (e.g., manure; 2-3 applications/crop season) and natural pesticides (e.g., tea saponins; 1 application/crop season) were used in organic farms. Synthetic nitrogen fertilizers (2-3 applications/crop season) and organophosphate pesticides (1 application/crop season) were used in conventional farms.]

[Arthropods were collected by sweep-netting the crop canopy 60 times along the farm ridges at four major rice crop stages (seedling, tillering, flowering, and ripening stage) during the first growing season (from April to July) in each study year. Samples were bagged, iced, and stored without chemical preservatives (e.g., ethanol) at −20ºC in the laboratory. Arthropods were identified to the finest taxonomic resolution possible and tallied under a dissecting scope.]

The percent forest cover around each study farm was estimated from Google Earth images by manually delimiting the forested areas within a 1-km radius buffer surrounding the farm and computing the fraction of these areas in the buffer zone.

*Stable isotope analysis of arthropod samples*

[Whole-body arthropods were oven dried at 50ºC for one week, pulverized, and weighed into tin capsules (5×9 mm). When necessary, several individuals of the same species were pooled into a single capsule to meet the minimum weight requirement for reliable results. Samples were analyzed at the UC Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The resulting carbon and nitrogen isotope ratios (δ13C and δ15N) were expressed in per mil (‰) relative to the international standards of Vienna PeeDee Beleminte for carbon and atmospheric N2 for nitrogen.]

*Arthropod trophic guild assignment*

[We adopted the concept of trophic guilds to examine community-level predator-prey trophic interactions in rice agro-ecosystems. Trophic guilds are aggregations of species that utilize similar dietary sources (i.e., occupy similar trophic niches) and constitute the basic components of food webs (Root 1967, Hawkins and Macmahon 1989). Moreover, they condense broad taxonomic information into distinct functional groups in communities (Dominik et al. 2018), rendering them appropriate for the purpose of this study.]

The arthropod samples were classified into four trophic guilds based on their dietary information and isotope signatures (Ecosphere paper citation): (1) “Predators” consisted of spiders and ladybeetles, which are the primary generalist arthropod predators inhabiting rice farms. (2) “Rice herbivores” consisted of major rice pests, including planthoppers, leafhoppers, and stink bugs. (3) “Tourist herbivores” consisted of herbivorous species without direct trophic association with rice plants, including grasshoppers and leaf beetles. (4) “Detritivores” consisted of arthropods that feed on decaying organic material or plankton, including various midge and fly species. Detailed information of the arthropod families/genera in each trophic guild is provided in Appendix Table S1. Because our study is mainly focused on the trophic interactions between generalist predators and their prey sources, we did not consider other trophic guilds (e.g., parasitoids) in the subsequent analyses.

*Data analysis*

To quantify the diet compositions of predators, we constructed a Bayesian stable isotope mixing model using the R MixSIAR package (Stock and Semmens 2017) to estimate the proportions of different prey sources (i.e., the three prey guilds including rice herbivores, tourist herbivores, and detritivores) in predators’ diet. Isotope data at the seedling stage for the three study years were omitted from the analysis due to insufficient sample sizes for model estimation. Individual farm-year and crop stage were included as fixed effects for predator isotope data; isotope data for the three prey guilds were pooled respectively to generate fixed source values in the model. Carbon and nitrogen concentration dependencies as well as the residual/process errors were incorporated to improve model estimates (Phillips and Koch 2002, Stock and Semmens 2016). Trophic discrimination factors (TDFs) were estimated from the diet-dependent discrimination equation proposed by Caut et al. (2009). Three Markov Chain Monte Carlo (MCMC) chains were run in the model, each with 50,000 iterations and a burn-in number of 25,000, along with a non-informative Dirichlet prior. Chain convergence was assessed via Gelman-Rubin and Geweke diagnostics. Bayesian posterior mean estimates were extracted for further analysis.

To examine how various factors may affect the pest consumption by predators, we first fit beta regression models with year, farm type, crop stage, percent forest cover, and the relative abundance of rice herbivores as fixed effects without interactions and the proportion of rice herbivores consumed in predators’ diet as the response using the R betareg package (Zeileis et al. 2016). We then refit the first model by adding the interaction terms among all the significant factors as the final model. Model parameters were estimated using maximum likelihood and the significance of factor terms were then analyzed by a two-way ANOVA (type II test) using the “Anova” function in the R car package (Fox and Weisberg 2018). Tukey’s post-hoc tests were performed for the significant factors using the “cld” function in the R emmeans package (Lenth and Lenth 2018). Because spiders and ladybeetles were the two most abundant generalist predator groups in our system and they exhibit distinct foraging behavior (e.g., sit-and-wait vs. active hunting) and microhabitat use, we also performed all the aforementioned analyses separately for each of the two predator groups. All analyses were conducted in R version 4.0.3 (R Core Team 2020).

**Appendix Table S1**. The arthropod families/genera in each trophic guild in this study.

|  |  |  |
| --- | --- | --- |
| Trophic guild | Order | Family/Genus |
| Predators | Araneae | Araneidae |
|  | Araneae | Clubionidae |
|  | Coleoptera | Coccinellidae |
|  | Araneae | Oxyopidae |
|  | Araneae | Tetragnathidae |
|  | Araneae | Thomisidae |
| Rice herbivores | Hemiptera | Alydidae/*Leptocorisa* |
|  | Hemiptera | Cicadellidae/*Nephotettix* |
|  | Hemiptera | Delphacidae/*Nilaparvata* |
|  | Lepidoptera | Hesperiidae |
|  | Hemiptera | Lygaeidae/*Pachybrachius* |
|  | Hemiptera | Pentatomidae/*Scotinophara* |
|  | Lepidoptera | Pyralidae |
|  | Orthoptera | Pyrgomorphidae/*Atractomorpha* |
| Tourist herbivores | Orthoptera | Acrididae |
|  | Coleoptera | Chrysomelidae |
| Detritivores | Diptera | Chironomidae |
|  | Diptera | Chloropidae |
|  | Diptera | Ephydridae |
|  | Diptera | Muscidae |
|  | Diptera | Sciomyzidae |
|  | Diptera | Stratiomyidae |
|  | Orthoptera | Tetrigidae |

**Results**

*Diet compositions of predators in rice farms*

Across organic and conventional farms during 2017-2019, the proportion of rice herbivores in all predators’ diet increased over the course of the crop season from ca. 25-40% at the tillering stage to over 87-94% at the ripening stage; the proportion of detritivores in predator’s diet decreased from ca. 45-55% at the tillering stage to 1-2% at the ripening stage; the proportion of tourist herbivores in the diet also decreased from ca. 16-20% at the tillering stage to 5-10% at the ripening stage (Fig. 1a).

Regarding individual predator groups, spiders and ladybeetles showed a marked difference in their diet compositions over crop stages during 2017-2019. Spiders consumed higher proportion of detritivores (ca. 40-50%) in their diet in the beginning of crop season (tillering stage) and substantially increased the consumption on rice herbivores up to 80-93% in late crop season (ripening stage) (Fig. 1b). On the contrary, ladybeetles in both organic and conventional farms steadily consumed a low proportion of detritivores (e.g., less than 10%) and over 82% of rice herbivores in their diet throughout the crop season (Fig. 1c). For both predator groups, tourist herbivores did not constitute an important prey source and contributed less than 25% to their diet (Fig. 1b, 1c).

*Patterns of rice herbivore consumption by predators*

We further analyzed rice herbivore consumption by predators since these herbivores are the main pests of concern. The patterns of rice herbivore consumption by all predators in organic and conventional rice farms were generally similar across the three study years, suggesting consistency in their feeding habits (Fig. 2). The consistency in herbivore consumption over years was also revealed by our beta regression model, which indicated that the proportion of rice herbivores consumed in all predators’ diet did not vary across years (*χ*2 = 1.99, *P* = 0.37; Table 1).

Interestingly, spiders and ladybeetles exhibited distinct within-season patterns of rice herbivore consumption. For spiders in organic and conventional farms, the proportion of rice herbivores in their diet increased toward later crop season, ranging from 25-41% (tillering) to 80-93% (ripening) (Fig. 2b), whereas for ladybeetles in in organic and conventional farms, the proportion of rice herbivores in their diet remained relatively stable throughout the season, ranging from 82-83% (tilling) to 94% (ripening) (Fig. 2c).

*Factors associated with rice herbivore consumption by predators*

The proportion of rice herbivores consumed in predators’ diet differed between organic and conventional farms (All predators: *χ*2 = 15.98, *P* < 0.001; Spiders: *χ*2 = 13.38, *P* < 0.001; Ladybeetles: *χ*2 = 6.70, *P* = 0.001; Table 1). In particular, all predators in conventional farms consumed higher proportion of rice herbivores in the diet compared with predators in organic farms (Tukey’s post-hoc test, *P* < 0.05; Table 2), although there was an interactive effect of farm type and year on spiders’ diet composition (*χ*2 = 7.64, *P* = 0.02; Table 2).

The proportion of rice herbivores consumed in predators’ diet also differed among crop stages (All predators: *χ*2 = 227.93, *P* < 0.001; Spiders: *χ*2 = 115.43, *P* < 0.001; Ladybeetles: *χ*2 = 152.60, *P* < 0.001; Table 1). Specifically, predators consumed higher proportion of rice herbivores in their diet at the flowering and/or ripening stage than they did at the tillering stage (Tukey’s post-hoc test, *P* < 0.05; Table 3).

Contrary to previous studies showing the importance of surrounding landscape in determining arthropod community structure and pest control by predators (Rusch et al. 2016) , but see Karp et al. (2018), the proportion of rice herbivores consumed in predators’ diet was not associated with the percent forest cover within a 1-km radius buffer surrounding the study farms (All predators: *χ*2 = 0.30, *P* = 0.58; Spiders: *χ*2 = 1.28, *P* = 0.26; Ladybeetles: *χ*2 = 0.77, *P* = 0.38; Table 1). Furthermore, the proportion of rice herbivores consumed was not associated with the relative abundance of rice herbivores in the field (All predators: *χ*2 = 0.36, *P* = 0.55; Spiders: *χ*2 = 1.38, *P* = 0.24; Ladybeetles: *χ*2 = 0.93, *P* = 0.33; Table 1).

Diet_proportion.tiff

Figure 1. The proportions (mean ± SE) of prey sources (rice herbivores, tourist herbivores, detritivores) consumed in predators’ diet in organic and conventional rice farms over crop stages. (a) All predators as a whole feeding guild; (b) spiders only; (c) ladybeetles only. The proportions are the posterior mean estimates from the Bayesian stable isotope mixing models averaged across the three study years.

Rice_herb_consumption.tiff

Figure 2. The proportion of rice herbivores consumed in predators’ diet in organic and conventional rice farms over crop stages in the three study years. (a) All predators as a whole feeding guild; (b) spiders only; (c) ladybeetles only. The proportions are the posterior mean estimates from the Bayesian stable isotope mixing models.

Table 1. Analysis of deviance (type II test) for the beta regression models fitted to data for all predators, spiders only, and ladybeetles only. The results were obtained from the Anova() function in the R “car” package (Fox and Weisberg 2018). For each model, interactions were tested only between significant factors.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model | Factor | *d.f.* | *χ*2 | *P* |
| All predators | Year | 2 | 1.99 | 0.37 |
|  | Farm type | 1 | 15.98 | <0.001 |
|  | Crop stage | 2 | 227.93 | <0.001 |
|  | Percent forest cover | 1 | 0.30 | 0.58 |
|  | Relative abundance of rice herbivores | 1 | 0.36 | 0.55 |
|  | Farm type × Crop stage | 2 | 2.06 | 0.36 |
| Spiders | Year | 2 | 7.92 | 0.02 |
|  | Farm type | 1 | 13.38 | <0.001 |
|  | Crop stage | 2 | 115.43 | <0.001 |
|  | Percent forest cover | 1 | 1.28 | 0.26 |
|  | Relative abundance of rice herbivores | 1 | 1.38 | 0.24 |
|  | Year × Farm type | 2 | 7.64 | 0.02 |
|  | Year × Crop stage | 4 | 0.99 | 0.91 |
|  | Farm type × Crop stage | 2 | 1.12 | 0.57 |
|  | Year × Farm type × Crop stage | 4 | 0.44 | 0.98 |
| Ladybeetles | Year | 2 | 13.20 | 0.001 |
|  | Farm type | 1 | 6.70 | 0.001 |
|  | Crop stage | 2 | 152.60 | <0.001 |
|  | Percent forest cover | 1 | 0.77 | 0.38 |
|  | Relative abundance of rice herbivores | 1 | 0.93 | 0.33 |
|  | Year × Farm type | 2 | 5.78 | 0.06 |
|  | Year × Crop stage | 4 | 6.80 | 0.15 |
|  | Farm type × Crop stage | 2 | 1.95 | 0.38 |
|  | Year × Farm type × Crop stage | 4 | 2.37 | 0.67 |

Table 2. Tukey’s post-hoc tests comparing the proportion of rice herbivores consumed in predators’ diet in organic and conventional rice farms. The tests were performed using the function cld() from the R “multcomp” package (Hothorn et al. 2008). Different superscript letters indicate significant differences in the means (α = 0.05) within each model.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model | Farm type | Mean (± SE) | Lower 2.5% | Upper 2.5% |
| All predators | Organic | 0.66a (± 0.015) | 0.63 | 0.69 |
|  | Conventional | 0.73b (± 0.014) | 0.70 | 0.76 |
| Spiders | Organic | 0.64a (± 0.020) | 0.60 | 0.68 |
|  | Conventional | 0.73b (± 0.019) | 0.69 | 0.77 |
| Ladybeetles | Organic | 0.86a (± 0.007) | 0.85 | 0.87 |
|  | Conventional | 0.88b (± 0.005) | 0.87 | 0.89 |

Table 3. Tukey’s post-hoc tests comparing the proportion of rice herbivores consumed in predators’ diet among the three crop stages (tillering, flowering, and ripening stage). The tests were performed using the function cld() from the R “multcomp” package (Hothorn et al. 2008). Different superscript letters indicate significant differences in the means (α = 0.05) within each model.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model | Crop stage | Mean (± SE) | Lower 2.5% | Upper 2.5% |
| All predators | Tillering | 0.32a (± 0.029) | 0.27 | 0.38 |
|  | Flowering | 0.84b (± 0.016) | 0.81 | 0.87 |
|  | Ripening | 0.92c (± 0.013) | 0.90 | 0.95 |
| Spiders | Tillering | 0.35a (± 0.036) | 0.28 | 0.42 |
|  | Flowering | 0.82b (± 0.021) | 0.78 | 0.86 |
|  | Ripening | 0.89b (± 0.024) | 0.84 | 0.93 |
| Ladybeetles | Tillering | 0.82a (± 0.012) | 0.80 | 0.85 |
|  | Flowering | 0.85a (± 0.007) | 0.84 | 0.87 |
|  | Ripening | 0.94b (± 0.004) | 0.93 | 0.95 |

**Discussion**

Key messages: 1) Consistent pest consumption over years: high potential in providing ecosystem service (biocontrol). 2) Different patterns between generalist predators: active vs. sit-and-wait. 3) Generalist predators (e.g., spiders) could be specialist when things matter (during high pest density) (context-dependent specialist)

* Summary of the main findings: (1) different dietary patterns of ladybeetles and spiders; (2) high consistency in pest consumption across the three study years for both predator groups; (3) factors that affect the proportions of rice herbivore consumed in predators’ diet
* Significance of this study: The first study to quantify the diet compositions of arthropod generalist predators in the field over three consecutive years as well as examine potential factors that may influence predators’ dietary patterns, providing more convincing evidence for the biocontrol potential of these predators

CK: also complement with molecular analysis (limitation: qualitative study [presence/absence], snap shot, etc.)

* Different dietary patterns of the two generalist predator groups: This may be due to the differences in their feeding modes (active-pursuit vs. sit-and-wait). Ladybeetles are active-pursuit feeders and may prefer rice pests to detritivores. In addition, they are more active in the upper part of the rice plant, where the relative abundance of rice herbivores to detritivores is higher compared to the lower part of the rice plant. On the other hand, most spiders in our study (Tetragnathidae) are orb weavers and thus their diet composition might reflect the relative abundances of the prey items in the surroundings. In fact, our data support this explanation that the relatively high consumption of detritivores and tourist herbivores by spiders at the tillering stage corresponds to the high relative abundances of these prey sources in the field
* High consistency in rice herbivore consumption of both predator groups across years: the feeding habits of generalist predators might not be as variable and unpredictable as previously thought, and therefore they could provide stable and reliable top-down control on pest herbivores
* Factors that affect the pest composition by generalist predators: possible explanations, comparisons with previous studies, implications for agricultural management
* Implication based on our results: For example, 1) farm type: overlooked ecosystem service by GAPs particularly in conventional farms, 2) crop stage: not much herbivores in early season (tillering, Cite our Ecosphere), much higher herbivore density later on > Therefore, farm management should avoid hurt GAP populations during sensitive / highly valuable stages.
* Limitations: (1) Not able to quantify the degree of intraguild predation, (2) Diet compositions reflects only the per capita effects of predator on prey, yet the overall effects of predators on prey populations also depend on the density of predators in the field

* Implications for agriculture: Our study provides evidence for consistent pest consumption by generalist predators, reducing previous concerns about whether generalist predators can exert effective top-down control on pest. Therefore, agricultural management should incorporate farming practices promoting arthropod generalist predators in the field to enhance biocontrol

**Acknowledgement**

We thank Miaoli District Agricultural Research and Extension Station for providing field assistance and logistic support. This study was funded by Council of Agriculture, R.O.C..

Reference

Albertini, A., S. Marchi, C. Ratti, G. Burgio, R. Petacchi, and S. Magagnoli. 2018. Bactrocera oleae pupae predation by Ocypus olens detected by molecular gut content analysis. BioControl **63**:227-239.

Bengtsson, J., J. Ahnström, and A. C. WEIBULL. 2005. The effects of organic agriculture on biodiversity and abundance: a meta‐analysis. Journal of Applied Ecology **42**:261-269.

Birkhofer, K., T. M. Bezemer, J. Bloem, M. Bonkowski, S. Christensen, D. Dubois, F. Ekelund, A. Fließbach, L. Gunst, and K. Hedlund. 2008a. Long-term organic farming fosters below and aboveground biota: Implications for soil quality, biological control and productivity. Soil Biology and Biochemistry **40**:2297-2308.

Birkhofer, K., A. Fließbach, D. H. Wise, and S. Scheu. 2008b. Generalist predators in organically and conventionally managed grass‐clover fields: implications for conservation biological control. Annals of applied biology **153**:271-280.

Birkhofer, K., A. Fließbach, D. H. Wise, and S. Scheu. 2011. Arthropod food webs in organic and conventional wheat farming systems of an agricultural long‐term experiment: a stable isotope approach. Agricultural and Forest Entomology **13**:197-204.

Boecklen, W. J., C. T. Yarnes, B. A. Cook, and A. C. James. 2011. On the use of stable isotopes in trophic ecology. Annual review of ecology, evolution, and systematics **42**:411-440.

Campbell, B. M., D. J. Beare, E. M. Bennett, J. M. Hall-Spencer, J. S. Ingram, F. Jaramillo, R. Ortiz, N. Ramankutty, J. A. Sayer, and D. Shindell. 2017. Agriculture production as a major driver of the Earth system exceeding planetary boundaries. Ecology and Society **22**.

Caut, S., E. Angulo, and F. Courchamp. 2009. Variation in discrimination factors (Δ15N and Δ13C): the effect of diet isotopic values and applications for diet reconstruction. Journal of Applied Ecology **46**:443-453.

Crowder, D. W., T. D. Northfield, M. R. Strand, and W. E. Snyder. 2010. Organic agriculture promotes evenness and natural pest control. Nature **466**:109-112.

Diehl, E., V. L. Mader, V. Wolters, and K. Birkhofer. 2013. Management intensity and vegetation complexity affect web-building spiders and their prey. Oecologia **173**:579-589.

Eitzinger, B., N. Abrego, D. Gravel, T. Huotari, E. J. Vesterinen, and T. Roslin. 2019. Assessing changes in arthropod predator–prey interactions through DNA‐based gut content analysis—variable environment, stable diet. Molecular Ecology **28**:266-280.

Eitzinger, B., and M. Traugott. 2011. Which prey sustains cold‐adapted invertebrate generalist predators in arable land? Examining prey choices by molecular gut‐content analysis. Journal of Applied Ecology **48**:591-599.

Fox, J., and S. Weisberg. 2018. An R companion to applied regression. Sage publications.

Geiger, F., J. Bengtsson, F. Berendse, W. W. Weisser, M. Emmerson, M. B. Morales, P. Ceryngier, J. Liira, T. Tscharntke, and C. Winqvist. 2010. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. Basic and Applied Ecology **11**:97-105.

Gomiero, T., D. Pimentel, and M. G. Paoletti. 2011. Is there a need for a more sustainable agriculture? Critical reviews in plant sciences **30**:6-23.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical Journal: Journal of Mathematical Methods in Biosciences **50**:346-363.

Huang, H., and P. Yang. 1987. The ancient cultured citrus ant. BioScience **37**:665-671.

Ingrao, A. J., J. Schmidt, J. Jubenville, A. Grode, L. Komondy, D. VanderZee, and Z. Szendrei. 2017. Biocontrol on the edge: Field margin habitats in asparagus fields influence natural enemy-pest interactions. Agriculture, Ecosystems & Environment **243**:47-54.

Karp, D. S., R. Chaplin-Kramer, T. D. Meehan, E. A. Martin, F. DeClerck, H. Grab, C. Gratton, L. Hunt, A. E. Larsen, and A. Martínez-Salinas. 2018. Crop pests and predators exhibit inconsistent responses to surrounding landscape composition. Proceedings of the National Academy of Sciences **115**:E7863-E7870.

Kehoe, L., A. Romero-Muñoz, E. Polaina, L. Estes, H. Kreft, and T. Kuemmerle. 2017. Biodiversity at risk under future cropland expansion and intensification. Nature Ecology & Evolution **1**:1129-1135.

Kuusk, A.-K., and B. Ekbom. 2012. Feeding habits of lycosid spiders in field habitats. Journal of Pest Science **85**:253-260.

Layman, C. A., M. S. Araujo, R. Boucek, C. M. Hammerschlag‐Peyer, E. Harrison, Z. R. Jud, P. Matich, A. E. Rosenblatt, J. J. Vaudo, and L. A. Yeager. 2012. Applying stable isotopes to examine food‐web structure: an overview of analytical tools. Biological Reviews **87**:545-562.

Lenth, R., and M. R. Lenth. 2018. Package ‘lsmeans’. The American Statistician **34**:216-221.

Michalko, R., S. Pekár, and M. H. Entling. 2019. An updated perspective on spiders as generalist predators in biological control. Oecologia **189**:21-36.

Muñoz-Cárdenas, K., F. Ersin, J. Pijnakker, Y. van Houten, H. Hoogerbrugge, A. Leman, M. L. Pappas, M. V. Duarte, G. J. Messelink, and M. W. Sabelis. 2017. Supplying high-quality alternative prey in the litter increases control of an above-ground plant pest by a generalist predator. Biological Control **105**:19-26.

Newton, J. 2016. Stable isotopes as tools in ecological research. eLS:1-8.

Phillips, D. L., and P. L. Koch. 2002. Incorporating concentration dependence in stable isotope mixing models. Oecologia **130**:114-125.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83**:703-718.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Roubinet, E., K. Birkhofer, G. Malsher, K. Staudacher, B. Ekbom, M. Traugott, and M. Jonsson. 2017. Diet of generalist predators reflects effects of cropping period and farming system on extra‐and intraguild prey. Ecological Applications **27**:1167-1177.

Rusch, A., R. Chaplin-Kramer, M. M. Gardiner, V. Hawro, J. Holland, D. Landis, C. Thies, T. Tscharntke, W. W. Weisser, and C. Winqvist. 2016. Agricultural landscape simplification reduces natural pest control: A quantitative synthesis. Agriculture, Ecosystems & Environment **221**:198-204.

Sih, A., P. Crowley, M. McPeek, J. Petranka, and K. Strohmeier. 1985. Predation, competition, and prey communities: a review of field experiments. Annual Review of Ecology and Systematics **16**:269-311.

Staudacher, K., O. Rennstam Rubbmark, K. Birkhofer, G. Malsher, D. Sint, M. Jonsson, and M. Traugott. 2018. Habitat heterogeneity induces rapid changes in the feeding behaviour of generalist arthropod predators. Functional ecology **32**:809-819.

Stiling, P., and T. Cornelissen. 2005. What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. Biological Control **34**:236-246.

Stock, B., and B. Semmens. 2017. MixSIAR GUI user manual v3. 1. San Diego.

Stock, B. C., and B. X. Semmens. 2016. Unifying error structures in commonly used biotracer mixing models. Ecology **97**:2562-2569.

Symondson, W., K. Sunderland, and M. Greenstone. 2002. Can generalist predators be effective biocontrol agents? Annual review of entomology **47**:561-594.

Wise, D. H., D. M. Moldenhauer, and J. Halaj. 2006. Using stable isotopes to reveal shifts in prey consumption by generalist predators. Ecological Applications **16**:865-876.

Zeileis, A., F. Cribari-Neto, B. Gruen, I. Kosmidis, A. B. Simas, A. V. Rocha, and M. A. Zeileis. 2016. Package ‘betareg’. R package **3**:2.

Citation for EC 2020:

COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE COUNCIL, THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE AND THE COMMITTEE OF THE REGIONS A Farm to Fork Strategy for a fair, healthy and environmentally-friendly food system COM/2020/381 final