**Running title**

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

**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

Agriculture is the largest land use type worldwide and the major driver for global biodiversity crisis and environmental degradation. Agricultural expansion and intensification have contributed to habitat loss and climate change, posing substantial threats on species and ecosystems (Kehoe et al. 2017). To mitigate such impacts, there is an urgent need for modern industrial agriculture to shift toward more ecological- and environmental-friendly practices (Gomiero et al. 2011).

Biological control by natural enemies constitutes an essential component of sustainable agriculture and has been increasingly applied in farm management to reduce the use of pesticides (Heimpel and Mills 2017). For herbivorous insect pests, two major groups of arthropod natural enemies are used as biocontrol agents: specialist (e.g., parasitoids) and generalist predators (e.g., spiders). It has been widely thought that specialists are more effective in pest control, as they are able to target specific pest species and thus minimize the undesirable non-target effect (Stiling and Cornelissen 2005). In contrast, generalist predators could feed on not only target pest but also alternative prey due to their polyphagous nature. As a result, studies have questioned the effectiveness of generalist predators as biocontrol agents (Symondson et al. 2002). Whether generalist predators are able to provide reliable top-down control on target pests is still a subject of much debate. Quantifying the diet compositions of these predators is a critical step toward assessing their biocontrol efficacy.

Empirical evidence for the biocontrol effectiveness of arthropod 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 abundance 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 abundance compared to specialists (Stiling and Cornelissen 2005). On the other hand, it has 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). Furthermore, these predators may interfere with each other or even engage in intraguild predation, disrupting the top-down control by other natural enemies (Prasad and Snyder 2006, Michalko et al. 2019).

Various local farm factors can influence the diet compositions of generalist predators in agro-ecosystems. The relative abundances of prey 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 feed continually on pest species even under increasing densities of alternative prey (Wise et al. 2006). Different farming practices can alter species compositions and densities in the field, which in turn affects the diet compositions of predators (Birkhofer et al. 2011). For instance, organic farming may promote prey diversity in the farms and thus increases predators’ diet breadths as a result of greater prey availability. In contrast, the application of synthetic chemicals may reduce the density of detritivores yet increase the abundance of certain pest herbivores (Birkhofer et al. 2008), potentially leading to higher consumption of these herbivores in predators’ diet. Moreover, arthropod communities may vary over the season through crop development, affecting predator-prey interactions and therefore the diet compositions of predators (Roubinet et al. 2017). Finally, surrounding landscape could alter predators’ foraging behavior and thus their diet by influencing the local species pool as well as the spatial distribution of predator individuals. Vegetation complexity has been shown to affect the prey capture rates in 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). Understanding how these aforementioned farm factors may affect the diet compositions of generalist predators is critical for evaluating the importance of these predators as biocontrol agents and can help design management schemes that enhance their biocontrol efficacy.

Climatic factors play a critical role in governing predator-prey interactions in arthropod communities (Schmitz and Barton 2014, Laws 2017). For example, temperature strongly influences the daily activity patterns of arthropods (Logan et al. 2006). Higher temperature can shift the activity timing or increase the activity levels of some prey species relative to others, potentially leading to a shift in predators’ diet towards those prey items with higher encounter rates. Furthermore, relative air humidity could alter the behavior or movement of arthropods due to water constraint and dehydration risks (Contreras et al. 2013, Vebrová et al. 2018), in turn affecting the vulnerability to predation. For example, if certain prey species spend more time in sheltering places or reduce their activity levels to avoid desiccation under low relative air humidity, then it would be more difficult for predators to encounter and capture these prey species. As temperature and moisture are the two key abiotic drivers for predator-prey trophic interactions and are projected to change dramatically in the future, it would be important to examine how the predators’ diets are associated with changes in temperature and humidity to better predict the biocontrol efficacy of these predators under future climate changes.

Using a stable isotope approach, this study aims to (1) quantify the diet compositions of two major groups of arthropod generalist predator (ladybeetles and spiders) in rice agro-ecosystems, (2) evaluate the annual variations in rice herbivore (pest) consumption by predators, and (3) examine how various biotic (relative abundance of prey items), farm-level (farm type, crop stage, and surrounding landscape), and environmental factors (degree days and daily mean relative humidity) are associated with the proportions of pest consumed in predators’ diet.

**Materials and Methods**

* Why we focused on spiders and ladybeetles for more detailed analysis (show abundance data to support our decision): the two most abundant GAP groups in our system, distinct foraging modes (active vs. sit-and-wait)

*Study system and experimental design*

*Sampling procedure and environmental data collection*

*Stable isotope analysis and arthropod trophic guild assignment*

*Data analysis (stable isotope mixing model and beta regression)*

**Results**

**Ecosphere 1) Raw data to create our main figure, 2) TIFF files for each figure.**

*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 (Figure 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) (Figure 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 (Figure 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 (Figure 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) (Figure 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) (Figure 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).

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), different from previous studies that reported the importance of surrounding landscape on arthropod community structure and biocontrol (citation). 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 et al. 2012). 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

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