**Running title**

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

**Abstract**

**Introduction**

* [Importance of biocontrol] 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] Besides specialist predators.., the potential of (underappreciated) generalist arthropod predators (GAPs) as biocontrol agents: traditional studies (cages, observations, etc.), molecular studies
  + Strength and limitation (qualitative data on diet composition = presence / absence of prey in GAPs’ diet) of molecular analysis
* [Knowledge gaps] To fully realized the potential of GAPs, we need to quantify their diet composition during crop growing season (crop stages), consistency over time (years), and factors affecting diet composition (biotic and abiotic).
  + Why need to consider crop stages
  + Why concern consistency
  + Why dissect biotic and abiotic factors > Provide insights (mechanisms) into understand, manage, and predict biocontrol service.
* Aim: 1) Quantify diet composition of GAPs over crop stages, 2) Examine the consistency of the diet composition over time (years). 3) Investigate how biotic and abiotic factors may affect the diet composition.
* Brief introduction of our system:
  + Stable isotope methods
  + Feeding guild (in order to introduce our GAP results).

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**

* Clarify why we focused on spiders and ladybeetles for more detailed analysis (show abundance data to support our decision)
* They happen to be active vs. sit-and-wait GAPs

*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 GAMM/GLMM)*

**Results**

* Overall (predator feeding guild) > Split to ladybeetles and spiders
* Temporal variation
  + Seasonal: Ladybeetles stable, Spiders increased toward late season (critical stage: flowering and ripening)
  + Yearly: consistent for each predator
  + GLM or 95% CI to support our key statement (consistent top-down effect [diet composition] over years)
* Biotic (relative abundance of rice herbivores, crop stage) and abiotic (farm type, landscape, degree days, humidity, year) factors affecting diet composition (focus on only a few key factors)
* Crop stage ~ plant H & Julian day. One solution is put plant H as biotic and Julian day as abiotic > If plant H has no effect, then crop stage may be mainly contributed by time (Julian day).

*Diet compositions of predators in rice farms*

Overall, both ladybeetles and spiders in organic and conventional rice farms consumed high proportions of rice herbivores in their diet, and the proportions increased over crop stage. In contrast, the proportions of detritivores and tourist herbivores consumed in predators’ diet decreased from tillering stage to flowering and ripening stage.

Regarding individual predator groups, ladybeetles showed generally high consumption of rice herbivores throughout the crop season, with the proportions increasing slightly over time; spiders consumed on average lower proportions of rice herbivores in their diet at tillering stage compared to ladybeetles, yet the proportions increased substantially at flowering and ripening stage, accounting for over 90% of their total diet.

*Annual variations in rice herbivore consumption by predators*

Despite some variations, both predator groups exhibited similar rice herbivore consumption patterns across the three study years, suggesting consistency in their respective feeding habits.

*Factors associated with the proportions of rice herbivore consumed in predators’ diet*

The proportions of rice herbivores consumed in ladybeetles’ and spiders’ diet both increased with the relative abundance of rice herbivores in the field, where the relationship is linear in ladybeetles (GLMM; *P* < 0.001) and non-linear in spiders (GAMM; *P* < 0.001).

Crop stage had a significant effect on the proportions of rice herbivores consumed in both ladybeetles’ and spiders’ diet (GLMM; ladybeetles: χ2 = 3724.0, df = 3, *P* < 0.001; spiders: χ2 = 569.6, df = 3, *P* < 0.001). Rice herbivore consumption was higher at flowering and ripening stage compared to tillering stage. Farm type was not associated with the proportion of rice herbivore consumed in ladybeetles’ diet (GLMM; χ2 = 0.38, df = 1, *P* = 0.54) but was marginally associated with the proportion of rice herbivore consumed in spiders’ diet (GLMM; χ2 = 3.28, df = 1, *P* = 0.07). Specifically, spiders in conventional farms consumed higher proportions of rice herbivores in their diet than spiders in organic farms. Landscape had no effect on the proportions of rice herbivore consumed in either ladybeetles’ or spiders’ diet (GLMM; ladybeetles: χ2 = 1.62, df = 2, *P* = 0.44; spiders: χ2 = 2.09, df = 2, *P* = 0.35).

The proportions of rice herbivores consumed in ladybeetles’ diet increased monotonically with degree days (DD) (GAMM; χ2 = 180.6, *P* < 0.001) and showed a non-linear relationship with daily mean relative humidity (GAMM; χ2 = 1429.6, *P* < 0.001). The proportions of rice herbivores consumed in spiders’ diet showed a nonlinear relationship with degree days (GAMM; χ2 = 237.7, *P* < 0.001) and decreased linearly with daily mean relative humidity (GAMM; χ2 = 6.48, *P* = 0.01).



**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).

* 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
* 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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Reference

Birkhofer, K., T. M. Bezemer, J. Bloem, M. Bonkowski, S. Christensen, D. Dubois, F. Ekelund, A. Fließbach, L. Gunst, and K. Hedlund. 2008. 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. 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.

Contreras, H. L., J. Goyret, M. von Arx, C. T. Pierce, J. L. Bronstein, R. A. Raguso, and G. Davidowitz. 2013. The effect of ambient humidity on the foraging behavior of the hawkmoth Manduca sexta. Journal of Comparative Physiology A **199**:1053-1063.

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.

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.

Heimpel, G. E., and N. J. Mills. 2017. Biological control. Cambridge University Press.

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.

Laws, A. N. 2017. Climate change effects on predator–prey interactions. Current Opinion in Insect Science **23**:28-34.

Logan, J. D., W. Wolesensky, and A. Joern. 2006. Temperature-dependent phenology and predation in arthropod systems. Ecological modelling **196**:471-482.

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

Prasad, R., and W. Snyder. 2006. Polyphagy complicates conservation biological control that targets generalist predators. Journal of Applied Ecology **43**:343-352.

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.

Schmitz, O. J., and B. T. Barton. 2014. Climate change effects on behavioral and physiological ecology of predator–prey interactions: implications for conservation biological control. Biological control **75**:87-96.

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.

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

Vebrová, L., A. van Nieuwenhuijzen, V. Kolář, and D. S. Boukal. 2018. Seasonality and weather conditions jointly drive flight activity patterns of aquatic and terrestrial chironomids. BMC ecology **18**:19.

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.