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Wanted dead or alive: Scavenging versus predation by three insect predators

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ABSTRACT

Many generalist insect predators engage in facultative scavenging. If an apparent predator frequently consumes dead prey instead of live prey then the biological control services provided by that predator may be overestimated. The use of unique protein markers on live and dead prey of the same species followed by gut content analysis of the predators is an effective method to distinguish between scavenging and predation events. The frequency of predation and scavenging on third instar *Lygus hesperus* (Hemiptera: Miridae) prey by *Collops vittatus* (Coleoptera: Melyridae), *Hippodamia convergens* (Coleoptera: Coccinellidae) and *Chrysoperla carnea* (Neuroptera: Chrysopidae) was measured using rabbit IgG and chicken IgG markers. Predators and rabbit IgG-marked dead (cadaver) and chicken IgG-marked live *L. hesperus* were placed on or adjacent to cotton plants enclosed in small cages for 6 h. The plants were then searched for all predators and uneaten prey and examined for the presence of the two proteins by IgG-specific enzyme linked immunosorbent assays (ELISA). The gut analyses revealed that scavenging was more prevalent than predation and that all three predators were facultative scavengers. In addition, direct visual observations of the predators in the cages and the number of *C. carnea* that went missing during the study suggested that *C. vittatus* and/or *H. convergens* were engaging in intraguild predation on *C. carnea*. When combined with a standard caging procedure the immunomarking procedure used here proved useful for distinguishing scavenging from predation events, which is not possible when using conventional prey-specific ELISA and PCR assays.

1. Introduction

Knowledge of the impact of arthropod predators on insect populations is crucial to understanding food webs in natural and managed ecosystems. The time required to gather direct observations of predation under natural conditions has constrained both the quantity and quality of data collected about arthropod predators and their prey consumption. Development of gut content assays using prey-specific protein and DNA markers to detect consumed prey was a significant step towards understanding what predators choose to eat under natural conditions (Greenstone and Morgan, 1989; Agustí et al., 1999; Chen et al., 2000). Yet to understand the impact that a specific predator species has on pest suppression requires more than just knowing what pests they eat. As the sophistication of gut content analyses increased, they not only gave more information about pest consumption but also highlighted other linkages between and within trophic levels (e.g. Harwood et al., 2001). Predator–prey relationships are not simple linear chains in most systems; they are tangled webs of interrelationships that include interguild predation, intraguild predation, cannibalism, and scavenging (Rosenheim et al., 1993; Mair and Port, 2001; Sentis et al., 2014).

The relationship between predation and scavenging has come under scrutiny recently because there is potential to overestimate the impact of an apparent predator if it consumes both dead and live individuals. Species-specific protein or DNA markers will not distinguish between scavenging and predation on the same prey species (Calder et al., 2005; Foltan et al., 2005). And yet this is the fundamental information required to understand the impact of a predator on its prey when that predator engages in both scavenging and predation (for convenience the term 'prey' refers here to both live and dead individuals). One approach has been to take two (presumably) similar prey species and then provide live prey of one species with dead prey of the second species. Predator gut contents were then analyzed for the presence of species-specific DNA markers (Heidemann et al., 2011; von Berg et al., 2012). This approach does give an indication of the predator's choices regarding dead versus live prey but inevitably these data are confounded: preference for dead versus live prey and preference for one species over the other. To avoid such confounding requires markers that can distinguish between individuals of the same species. Protein markers can be applied to prey items that are then released into cages or field plots (Hagler, 2011; Mansfield et al., 2008). The use of such markers on live and dead prey of the same species followed by gut content analysis provides an effective method to distinguish scavenging from

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predation. Proof of this concept was demonstrated by Zilnik and Hagler (2013) using simple laboratory arenas containing a single predator with one live and one dead prey item of the same species.

The aim of this study was to measure the frequency of predation and scavenging when predators were given the choice of live and dead prey items of the same species in a more complex environment. Live and dead *Lygus hesperus* Knight (Hemiptera: Miridae) prey were marked with different protein markers so that predation and scavenging events could be identified by protein-specific ELISAs. The complexity of the environment was increased (compared with Zilnik and Hagler, 2013) by using potted cotton plants in cages as the experimental arena and by placing three predator species in each cage with one live and one dead prey item. The use of cages made it possible to collect predators and uneaten prey for ELISAs at the end of the experiment. The transparent cages also allowed us to directly observe, to a certain degree, some of the predator activity occurring in the cages. The targeted prey for this study was *L. hesperus*, a notorious omnivorous pest of cotton and many other crops. The predators were adult *Collops vittatus* (Say) (Coleoptera: Melyridae), adult *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) and larval *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). These predators have been identified as "key" predators of *L. hesperus* in the cotton agroecosystem (Hagler and Blackmer, 2013).

2. Methods

2.1. Predator collection and maintenance

Field collections and experiments were conducted in July 2013 and June–July 2014. Insects used in this study were collected with a sweep net from an alfalfa field at the Maricopa Agricultural Center located near Maricopa, Arizona, USA (GPS coordinates: 33° 04′ 37″ N, 111° 58′ 26″ W). Field collected adult *C. carnea* were then reared in the laboratory using the method described by Ridgway et al. (1970). The *C. carnea* used in the feeding studies were 2.0 to 3.0 mm long, third instar larvae drawn from the culture for experiments as needed.

Adult *C. vittatus* and *H. convergens*, and *C. carnea* larvae were kept in plastic containers with mesh panels in the lids (2.8 L sterilite containers) under laboratory conditions (27 °C, 20% RH) until used in experiments. These predators were supplied *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) eggs as food ad libitum and water on moistened sponges.

2.2. Prey marking procedure

Live and dead (cadaver) *L. hesperus* were marked both internally and externally when presented to the predators. The use of both internal and external marks was appropriate because chewing predators (the beetles) and sucking predators (the lacewing larvae) acquire marks differently (Hagler et al., 2015a). Third instar *L. hesperus* serving as the cadaver treatment were taken directly from the field collections described above and for at least 24 h were offered green beans soaked in rabbit IgG solution (1 mg/mL, Sigma Chemical Co. No. I5006; Saint Louis, MO, USA) to ingest the internal marker. They were then killed by freezing at −80 °C overnight. Next, the cadavers were placed in a clean Petri dish and marked topically using a disposable pipette with rabbit IgG (10 μL of 1.0 mg/mL dilution). Live third instar *L. hesperus* were taken from a laboratory culture reared on an artificial diet that contained, in part, raw chicken eggs (Debolt, 1982). As such, these *L. hesperus* readily obtained an internal chicken IgG mark by feeding on the artificial diet. These nymphs were chilled briefly to immobilize them and then topically marked as described above with chicken IgG (10 μL of 1.0 mg/mL dilution, Sigma Chemical Co., No. I-4881, St. Louis, MO, USA). Live and dead prey items were externally marked fresh each day before use in all experiments. Unmarked *L. hesperus* nymphs from the field collections were frozen at −80 °C as negative controls.

2.3. Detection interval of marked prey after consumption

The detection period for live *L. hesperus* marked with rabbit or chicken IgG is at least 6 h (Hagler, 2011; Hagler et al., 2015a) but equivalent data for scavenging on marked *L. hesperus* cadavers was not available. To determine the detection period for scavenging, adult *C. vittatus* were offered *L. hesperus* cadavers that had been externally marked as described above with rabbit and chicken IgG. The beetles were monitored closely at 25 °C until a single cadaver was consumed. Individual beetles were then frozen at −80 °C at 0, 3, 6, or 12 h after consumption. The beetles were assayed for the presence of both rabbit and chicken IgG using the sandwich ELISAs described by Hagler (2011). Predators that had not been fed a protein marked prey item were also frozen at −80 °C as negative controls.

2.4. Detection of scavenging and predation in choice tests

Prey choice tests were conducted in 30 × 30 × 30 cm cages (BugDorm1, MegaView Science Co Ltd., Taichung, Taiwan) in a climate controlled glasshouse (21–35 °C, 30% RH). Five identical caged arenas were erected each day and the experiment was repeated until 100 replicates (caged plants) had been completed. Each cage contained one cotton plant (Bayer CropScience ST5458B2F) in a square pot (10.2 × 10.2 × 10.2 cm). Plants were selected to be as tall as possible (e.g., ca. 20 cm) without touching the top or sides of the cage. Plant leaf surface area was approximately 101 ± 6 cm^2 (mean ± SE, n = 17 plants measured using a LiCor LI-3100C). Parafilm M® (Bemis Flexible Packaging, Oshkosh, WI, USA) was wrapped across the top of the pot, over the soil surface and around the plant stem. This provided a smooth surface on which to place the cadaver and minimized the opportunity for live prey or predators to burrow into the soil.

Into each cage, one live chicken IgG-marked *L. hesperus* nymph was released first and placed directly onto the plant. The nymph was given 15 min to settle on the plant before releasing one individual of each predator (*C. vittatus*, *H. convergens* and *C. carnea*) onto the plant. The rabbit IgG-marked *L. hesperus* cadaver was placed into the cage last on the Parafilm cover at the base of the plant. After 6 h, the predators were hand collected, placed into an individual 1.5 mL tube, and frozen at −80 °C. Any predation or scavenging events observed while collecting the predators were recorded. Each individual predator was assayed for the presence of rabbit and chicken IgG marks using protein-specific sandwich ELISAs (Hagler, 2011). A predator was scored positive for the presence of the respective marks if its ELISA value was greater than the mean absorbance + 3 SD of the negative controls tested for each predator (Table 1). As an additional check, the plant and cage were searched thoroughly to find the live and cadaver prey items. The living and cadaver *L. hesperus* that were recovered after exposure to the predators were also assayed by each ELISA to confirm presence of the targeted mark (positive controls) and to check for cross-contamination. Again, the detection threshold was the mean absorbance + 3 SD of unmarked *L. hesperus* (Table 1). The recovery of live prey and cadavers from the cages along with incidental visual observations of predator behavior were compared with the ELISA results to help interpret the data. The proportion of scavenging (rabbit IgG) and predation (chicken IgG) events detected in the predator gut contents was

Table 1
Mean (+SD) ELISA absorbance values for unmarked insects and detection thresholds (mean + 3 SD) for the chicken and rabbit IgG markers.

Species	n	Chicken IgG		Rabbit IgG	
		mean + SD	Threshold	mean + SD	Threshold
L. hesperus	29	0.047 + 0.008	0.072	0.042 + 0.008	0.066
C. vittatus	27	0.046 + 0.007	0.068	0.053 + 0.016	0.100
H. convergens	20	0.045 + 0.006	0.063	0.046 + 0.011	0.078
C. carnea	22	0.048 + 0.009	0.075	0.040 + 0.006	0.057

compared using z-tests conducted in Sigmaplot version 13 (Glanz, 1997).

3. Results

3.1. Detection interval of marked prey after consumption

Both protein marks were detected in 85 to 95% of *C. vittatus* specimens that were assayed 0, 3 and 6 h after feeding on a protein-marked *L. hesperus* cadaver (n = 20 for each time interval, Fig. 1). Detection declined to 20 to 40% at 12 h after consumption (n = 5, Fig. 1). This indicated that any predation or scavenging events during the 6 h choice tests were likely to be detected.

3.2. Recovery of live prey and cadavers

Overall, 90 chicken IgG-marked *L. hesperus* were recovered (87 alive and 3 dead) after the 6-hour exposure period. Of these, 80 (89%) tested positive for the presence of the chicken protein marker (Table 2). One *L. hesperus* was negative for chicken IgG but a predator from the same cage tested positive for this marker, suggesting a false negative result for the prey. One chicken IgG-marked *L. hesperus* also tested positive for rabbit IgG. This individual either engaged in scavenging, had some other contact with the rabbit IgG-marked cadaver, or was a false positive. Six of the 10 missing prey were identified in the gut contents of a predator recovered from the same cage. The other four cages that were missing a live *L. hesperus* were also missing the *C. carnea* (see Section 3.3).

A total of 75 rabbit IgG-marked *L. hesperus* cadavers were recovered from the cages after the 6 hour exposure period (Table 2). Of these, 71 (95%) tested positive for the presence of the rabbit protein marker. One cadaver was negative for rabbit IgG but tested positive for chicken IgG. Presumably, this was a false negative from the cadaver because one of the predators from the same cage did test positive for rabbit IgG. The chicken IgG-marked *L. hesperus* was not recovered from this cage nor was it detected in gut contents so it is not clear how the chicken IgG mark was transferred to the cadaver. Of the 25 missing cadavers, 14 were identified in the gut contents of a predator recovered from the same cage. In three cages both the cadaver and a predator (two *C. carnea* and one *C. vittatus*) were missing but the other eight cadavers could not be accounted for. These missing prey were either lost within the cage due to their small size, or were not detected in the predators that had consumed them (i.e., false negatives).

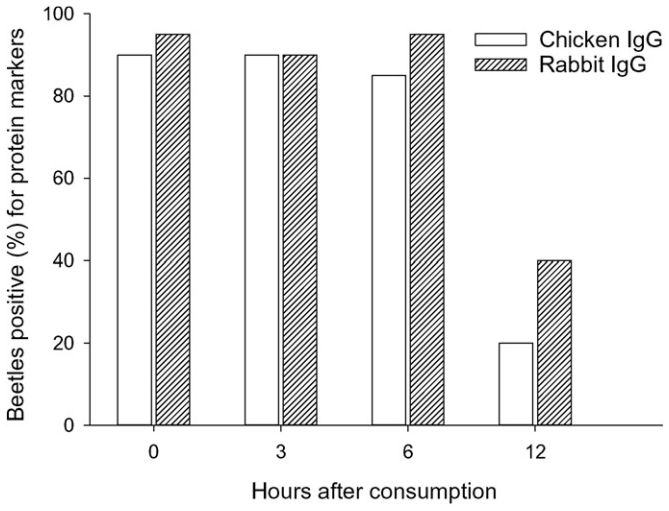

Fig. 1. Percentage of *Collops vittatus* that tested positive for chicken IgG and rabbit IgG after consuming one *Lygus hesperus* cadaver treated with both markers.

3.3. Detection of scavenging and predation in choice tests

Almost all *C. vittatus* (n = 95) and *H. convergens* (n = 100) were recovered from the cages after the 6-hour exposure period, but only two-thirds of the *C. carnea* were recovered (n = 67, Table 2). The rabbit IgG marker indicating scavenging was detected significantly more often in predator gut contents (n = 44, Tables 2 and 3) than the chicken IgG marker that indicated predation (n = 15, z = 3.14, P = 0.002). Scavenging was detected most often in *C. vittatus* (26% of individuals recovered, Table 2) and *C. carnea* (22%) but only occasionally in *H. convergens* (4%). Equal numbers of individuals tested positive for predation in each species (5% of *C. vittatus* and *H. convergens*, 7% of *C. carnea*). Too few predators tested positive for the protein markers to permit statistical comparisons between species. There were five individuals that tested positive for both predation and scavenging (two *C. vittatus*, two *C. carnea*, and one *H. convergens*, Table 3). There were also five cases where two predators in the same cage tested positive for scavenging on the same cadaver (three *C. vittatus* + *C. carnea* and two *C. vittatus* + *H. convergens*).

Just over half of the IgG marker detections (32 out of 59) were confirmed either from missing *L. hesperus* or from observed evidence of feeding (Fig. 2). Of the unconfirmed detections, nine were predation and 18 were scavenging events. The majority of scavenging and predation events by *C. vittatus* were confirmed (22 out of 30 events) in contrast to *H. convergens* and *C. carnea*, with only five confirmed events each (Fig. 2). For *C. carnea*, all confirmed events were for scavenging only. In one cage the *C. carnea* was seen feeding on the *L. hesperus* nymph but the prey was not wholly consumed. That predator did not test positive for the chicken IgG marker so the event was not detected nor included in the predation count.

One-third of the *C. carnea* larvae were not recovered at the end of the 6 hour exposure period. A *C. vittatus* was seen eating a *C. carnea* in one cage. In another cage, the *C. carnea* was seen feeding on the *L. hesperus* cadaver before both disappeared, and subsequently the *C. vittatus* in that cage tested positive for scavenging. In seven cages both the *L. hesperus* cadaver and the *C. carnea* were missing and the *C. vittatus* tested positive for scavenging. These observations suggest that *C. vittatus* occasionally consumed the *L. hesperus* cadaver and the *C. carnea* larva.

4. Discussion

4.1. Marker performance

Detection of the markers on the prey items and retention of the markers within *C. vittatus* gut contents were similar to results of previous studies on the same prey and/or same predators (Hagler, 2011; Hagler et al., 2015a). Of the 164 marked *L. hesperus* prey recovered from the cages, only two (1.2%) showed evidence of cross-contamination (i.e., they were positive for the IgG marker that they were not marked with). Previous immunomarking studies have shown there is about a 1.0% assay error rate associated with this procedure (Hagler et al., 2014, 2015b). We have attributed this to human error (e.g., day-to-day variation) when conducting the assays. It is also possible that the protein marks could be inadvertently transferred between prey if a predator contacted both prey items. However, this is unlikely because none of the predators assayed from the two relevant cages yielded a positive reaction for the transferred mark. Incidental contact, via a non-predation event, between the live and cadaver *L. hesperus* is also possible. It seems most plausible that the protein mark was transferred while a live *L. hesperus* was scavenging on the cadaver because *L. hesperus* is a known omnivore (Hagler and Blackmer, 2013).

We did document a clear example of a false negative ELISA result. Specifically, an ELISA conducted on an individual predator that was observed feeding on a protein-marked *C. carnea* larva failed to detect the marker. It should be noted however, that this predator only partially

Table 2
Predators and prey recovered after 6 h in the cages and frequency of IgG marker detection (n = 100).

Number of individuals	Live *L. hesperus* chicken IgG marker	Dead *L. hesperus* rabbit IgG marker	*C. vittatus*	*H. convergens*	*C. carnea*
Found alive	87	–	93	98[b]	64
Found dead	3	75[a]	2	2	3
Missing	10	25	5	0	33
Positive chicken IgG	80	1	5	5	5
Negative chicken IgG	10	73	90	94	62
Positive rabbit IgG	1	71	25	4	15
Negative rabbit IgG	89	3	70	95	52

[a] One cadaver was broken accidentally so was not tested for IgG markers.
[b] One beetle escaped during the collection process so was not tested for IgG markers.

consumed the prey. In all likelihood the predator did not consume enough of the marked prey for detection by ELISA (see Hagler et al., 2015b). Future prey immunomarking quality control studies are warranted to determine the frequency with which protein markers transfer to predators that only partially consume their prey.

A surprising outcome from our study was that a relatively high proportion (46%) of predators yielded a positive assay response for the marked prey, but the relevant prey item in each cage appeared intact with no visual evidence of attack. There is a slight risk of accidental cross-contamination during sample collection if the predator and the marked prey are collected together (e.g., in a sweep net, King et al., 2008; Greenstone et al., 2011; Hagler et al., 2014). However, this was unlikely in this case because the predators and marked prey items were handled individually with clean forceps. Partial consumption of the rabbit IgG-marked cadaver might be sufficient to transfer the rabbit IgG marker without leaving obvious traces of feeding damage because the cadavers were too fragile to withstand close examination when recovered from the cages. When the chicken IgG marker was detected in a predator, yet the live *L. hesperus* nymph was recovered unharmed, contact during a failed predation attempt may have been enough to transfer the mark to the predator. Previous studies have shown that many predation attempts are unsuccessful. Noriyuki et al. (2011) reported a wide range of successful predation events for coccinellids attacking different aphid species (e.g., from <20% to >80%). Prey characteristics such as size, hairiness and defensive behavior can influence predation success (Combes et al., 2013; Korenko et al., 2014; Sugiura and Yamazaki, 2014). In this study, variation was minimized because only one prey species was offered and the prey were of the same size. Clearly, future studies are needed to determine the likelihood of protein marker transfer during an unsuccessful predator attack (e.g., a predator probes, bites, or grasps, but does not kill its prey) on various types of prey species. The effect of external versus internal marking on marker transfer during unsuccessful attacks also warrants investigation. Immunomarking techniques may offer a new way to quantify unsuccessful attacks by predators that complement direct observations.

4.2. Predation and scavenging on L. hesperus

Scavenging on cadaver *L. hesperus* occurred more frequently than predation on the live nymphs, and *C. vittatus* was the dominant scavenger of the three predators examined. Previous laboratory choice tests revealed that *C. vittatus* strongly preferred cadavers of whiteflies, lacewing larvae, and *L. hesperus* nymphs over their live counterparts. Also, *C. vittatus* has been directly observed in the field readily feeding on *H. convergens* cadavers (Zilnik and Hagler, 2013). Our study and others suggest that scavenging may be the dominant feeding mode for *C. vittatus*. Scavenging by *C. carnea* occurred three times more frequently than predation, but the number of predation and scavenging events attributed to *C. carnea* was probably an underestimate because only two-thirds of the larvae were recovered from the cages (see Section 4.3 for discussion of intraguild predation). In contrast to the other two predators, *H. convergens* engaged equally in scavenging and predation. Scavenging is rarely reported among coccinellids, unlike cannibalism and intraguild predation which occur frequently (Hodek and Honêk, 1996; Burgio et al., 2002). Moser et al. (2010) showed that starved coccinellid larvae were more likely to scavenge than satiated larvae, so scavenging may be a response to a limited supply of live prey.

Few predators fed more than once, with just five individuals testing positive for both scavenging and predation events on *L. hesperus*. Occasionally, two predators fed upon the same cadaver, presumably because the first predator only partially consumed the marked prey item and then the remains were eaten by another predator. In such instances, direct observation of predation is the only method that can detect which predator fed first.

Table 3
Number of predators that tested positive for scavenging, for predation, or for both feeding modes.

Predator species	Scavenging (rabbit IgG)	Predation (chicken IgG)	Both (rabbit + chicken IgG)	Total
C. vittatus	23	3	2	28
H. convergens	3	4	1	8
C. carnea	13	3	2	18
Total	39	10	5	54

4.3. Intraguild and secondary predation

Intraguild predation occurs frequently among generalist insect predators and it is well documented that lacewing larvae are vulnerable to predation by higher tiered predators (Rosenheim et al., 1993; Hagler and Blackmer, 2015). The missing *C. carnea* larvae were presumably eaten by one of the beetle predators. In fact, one *C. vittatus* was observed engaging in intraguild predation on *C. carnea*. A *C. carnea*-specific PCR assay, such as the one used by Hagler and Blackmer (2015), could be used in concert with immunomarking assays to confirm predation events on *C. carnea*. Alternatively, the immunomarking procedure used here could be modified to study both intraguild predation and cannibalism on *C. carnea*. For instance, we have modified the procedure to simultaneously examine the degree of intraguild predation and cannibalism occurring on rabbit IgG and chicken IgG marked second and fifth instar *C. carnea*, respectively (JRH, unpublished data).

Secondary predation is a source of error that can confound the results of all types of molecular gut analysis, e.g., prey-specific ELISA, PCR, and prey immunomarking (Calder et al., 2005; Foltan et al., 2005). Secondary predation occurs when a higher tiered predator consumes a lower tiered predator that had previously consumed the targeted prey (Harwood et al., 2001). Direct observations combined with rabbit IgG marker detection confirmed one example in this study that could be termed 'secondary scavenging' because the predator *C. vittatus* consumed a *C. carnea* larva that had already eaten the *L. hesperus* cadaver. There were seven cases where, in the absence of direct observation, it was impossible to distinguish secondary scavenging from distinct incidents of scavenging on *L. hesperus* and predation on *C. carnea* by the same predator, *C. vittatus*. Predation on live *L. hesperus*

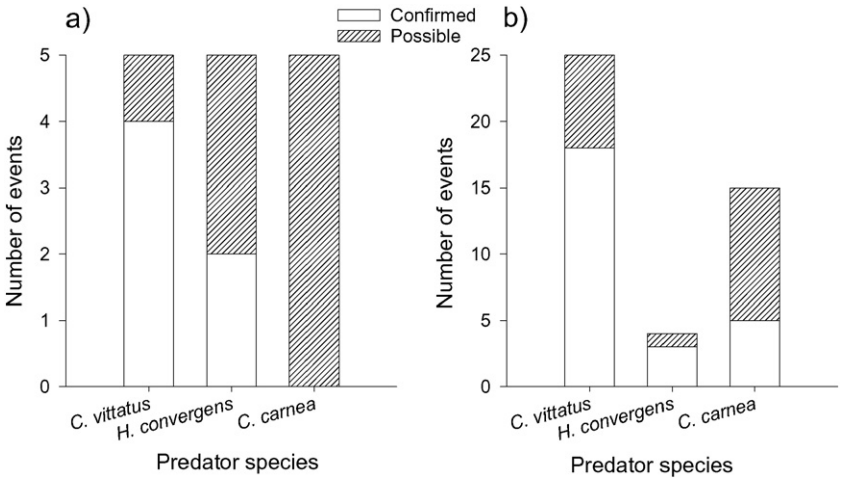

Fig. 2. Number of confirmed and possible events of a) predation and b) scavenging on *Lygus hesperus* by the predators *Collops vittatus*, *Hippodamia convergens* and *Chrysoperla carnea* (note the change in scale on the y-axes). Live and dead prey were treated with unique protein markers. An event was confirmed when a predator tested positive for a protein marker and the *L. hesperus* was missing or evidence of feeding was visible at the end of the experiment. An event was deemed possible when a predator tested positive for a protein marker but the prey was recovered alive or no feeding was evident.

occurred less frequently than scavenging and there were no potential examples of secondary predation identified based on the detections of the chicken IgG marker. The duration of these cage experiments was kept short at 6 h to maximize the probability of detecting predation/scavenging events. This means the probability of detecting secondary predation/scavenging was also relatively high because the duration of the experiments closely matched the gut retention period of the markers.

4.4. Describing food webs using unique protein markers

In summary, no single method for detecting predation is capable of addressing all possibilities in complex food webs. Prey-specific protein and DNA markers can detect prey consumption under natural conditions but cannot distinguish between scavenging and predation so predation rates may be overestimated. The use of unique protein markers on live and dead prey distinguishes effectively between scavenging and predation (as this study clearly shows), however the mark and release process used on the prey requires some modification of the natural environment during field experiments. The appropriate method depends on the hypothesis to be tested for every study. The advantages and disadvantages of each method need to be considered in relation to the hypothesis and the ecosystem in question. Ideally, a combination of gut assay methods would produce more informative results than any single method.

The protein immunomarking procedure combined with the caging procedure used here facilitated direct visual observation, and demonstrated that scavenging occurred more frequently than predation. Our study showed that all three predator species were facultative scavengers. Indeed one species, *C. vittatus*, may be better described as a scavenger that engages in facultative predation. Complications arose because at least one of the three predators apparently engaged in intraguild predation and secondary scavenging. These additional interactions were inferred based on the insects recovered from the cages (e.g., there were many *C. carnea* missing) at the end of the experiments and direct observations of insect predation within the cages. Direct observations of predation are time consuming but remain a valuable method to disentangle complex predator–prey interactions (Morris et al., 2002). Direct observations are particularly valuable where there are multiple types of interactions occurring within the same food web such that additional information is needed to interpret accurately the results obtained from gut content analysis. Moreover, unlike direct field observations of predation, the direct observations made from the perimeter of a caged arena are not disruptive to the insect's normal foraging processes and are relatively easy to obtain. Future studies will use this methodology in an even more realistic environment. Specifically, we plan to conduct whole plant field cage studies such as those described by Hagler (2011) to examine scavenging activity of the entire cotton arthropod predator complex. Successful application of immunomarking methods to measure predation and scavenging under natural conditions is the next step to unravel food webs in agroecosystems.

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