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Choosing natural enemies for conservation biological control: use of the prey detectability half-life to rank key predators of Colorado potato beetle

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Abstract

Determining relative strengths of trophic links is critical for ranking predators for conservation biological control. Molecular gut-content analysis enables ranking by incidence of prey remains in the gut, but differential digestive rates bias such rankings toward predators with slower rates. This bias can be reduced by indexing each predator's half-life to that of the middle-most half-life in a predator complex. We demonstrate this with data from key species in the predator complex of Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), comprising adults and immatures of four taxonomically diverse species. These animals display order-of-magnitude variation in detectability half-life for the cytochrome oxidase I DNA sequence of a single CPB egg: from 7.0 h in larval *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae) to 84.4 h in nymphal *Perillus bioculatus* (Fabricius) (Hemiptera: Pentatomidae). The raw species-specific incidence of *L. decemlineata* DNA in the guts of 351 field-collected predators ranged from 11 to 95%, ranking them as follows: *C. maculata* adults < *Lebia grandis* Hentz (Coleoptera: Carabidae) adults < *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) adults < *P. maculiventris* nymphs < *P. bioculatus* adults < *P. bioculatus* nymphs. Half-life adjustment reorders the rankings: *C. maculata* adults < *P. bioculatus* adults < *P. bioculatus* nymphs < *P. maculiventris* nymphs < *L. grandis* adults < *P. maculiventris* adults. These changes in status demonstrate the value of half-life-adjusted molecular gut-content data for ranking predators. This is the first study to measure prey detectability half-lives for the key arthropod predators of a major insect pest, and to use them to evaluate the relative impact of all adults and immatures in this predator complex.

Introduction

Conservation biological control consists of 'premeditated actions for protecting and maintaining natural enemies' (Rabb et al., 1976). In practice, it comprises modifications of crop cultural practices (Landis et al., 2000) and/or of pest management tactics (Gurr et al., 2000) to attract, arrest, and protect natural enemies. Most insect pests are attacked by a variety of natural enemies, not all of which are equally effective in pest suppression (Loreau et al., 2001; Straub & Snyder, 2006). This leads to the question of how one determines which specific natural enemies should be conserved, and, given several promising candidates, what is the relative return on investment for each species added (Cardinale et al., 2003). Agroecosystems can be surprisingly complex and speciose (Greenstone & Sunderland, 1999), making this a non-trivial question.

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Both the number and identity of species within a predator complex can determine the level of prey suppression (Root, 1973; Snyder et al., 2004; Ives et al., 2005). Predator feeding behavior can be complex (Sunderland, 1996, 1999; Juen & Traugott, 2005; Otto et al., 2008). For example, many predators are not strictly predaceous, acquiring nutrients from a variety of plant tissues, including pollen, seeds, and floral and extrafloral nectars, as well as honeydew (Lundgren, 2009). Further, the impact of plant resources on predator effectiveness may depend not only on their absolute availability, but also on their dispersion in the field and location on plants (Andow & Risch, 1985; Straub & Snyder, 2008). In mixed-cropping systems, the identity and arrangement of plants may also enhance or reduce the attractiveness of plant kairomones (Greenstone & Dickens, 2005). Finally, polyphagous predators tend to enter and remain in the system as long as alternate prey are available (Harwood et al., 2007; Birkhofer et al., 2008), but stenophages may fail to appear unless the pest is present in abundance (Chang & Kareiva, 1999; Symondson et al., 2002), making it difficult to attract what appear at face value to be excellent candidates.

Predation is the most difficult interspecific interaction to study in the field (Sunderland, 1988; Greenstone & Morgan, 1989). Of the available approaches (reviewed in Symondson, 2002), gut analysis is least disruptive to ecosystem processes: the field is visited intermittently, and collected predators are assayed in the laboratory. Many arthropod predators are fluid feeders, so a molecular approach is usually necessary to assess all interactions in a predator–prey system. Two technologies have dominated molecular gut-content analysis: serology, with monoclonal antibody-based assays predominating (Greenstone, 1996; Harwood et al., 2004), and polymerase chain reaction (PCR)-amplification of prey deoxyribonucleic acid (DNA) sequences (Symondson, 2002; Sheppard & Harwood, 2005; Gariepy et al., 2007).

Because predators are trophically versatile, remains detected in the gut of a particular predator may not reflect predation by that predator on a live prey item, but rather scavenging on an already-dead animal, or it may reflect secondary predation on another predator that had killed and consumed that prey item (Harwood et al., 2001; Calder et al., 2005; Foltan et al., 2005; Juen & Traugott, 2005; Sheppard et al., 2005). Even when such 'food-chain errors' (Sunderland, 1996) are not an issue, so that an assay positive does represent predation, interpretation of assay data is not straightforward (Sopp & Sunderland, 1987). In the simple case where an assay positive represents the remains of a single prey item, the detectability of prey material within predators diminishes as it is digested (Hosseini et al., 2008, and references therein). Because the incidence of positives in a field collection is an instantaneous snapshot of an hours-long feeding and digesting process, predator species with long detectability intervals for prey material will display a higher incidence of prey remains in the gut than those having shorter detectability intervals but identical feeding histories.

Exponential and binary regression models for decay of detectability with time since cessation of feeding on a standard meal provide good fits to molecular gut-content assay data (Greenstone & Hunt, 1993; Hagler & Naranjo, 1997; Chen et al., 2000; Ma et al., 2005; Greenstone et al., 2007; Fournier et al., 2008; Hosseini et al., 2008). Binary regression models are particularly appropriate for frequency data, such as those arising from gut-content analysis. Hence, the detectability half-life, defined as the time after which only half of the target meals can be detected in a cohort of predators as estimated by probit analysis, is an appropriate index of the detectability interval (Chen et al., 2000). Given a single-prey multiple-predator system, an index of each predator's half-life adjusted to that of the middle-most half-life in the predator complex can be used as a first-approximation adjustment to raw frequencies of prey remains in the guts of all predators. We illustrate this approach with a system in which a significant but manageable number of species and stages compose the complex of important predators of a single pest.

Several generalist and stenophagous arthropod predator species have been observed feeding on the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), in eastern North America (Ferro, 1994); of these, four were most abundant in our collections and are most prominent in the literature. The spotted pink lady beetle, *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae), is often the most abundant generalist predator on potato foliage (Benton & Crump, 1981; Hazzard et al., 1991; Hilbeck et al., 1997) and may kill more than half the *L. decemlineata* eggs in a generation (Groden et al., 1990; Hazzard et al., 1991), as well as many small larvae. The spined soldier bug, *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), is a common generalist in potato (Hazzard et al., 1991; Heimpel & Hough-Goldstein, 1992; Ferro, 1994), consuming *L. decemlineata* eggs and larvae (Hough-Goldstein & McPherson, 1996; Tipping et al., 1999). The ground beetle *Lebia grandis* Hentz (Coleoptera: Carabidae) is an extreme specialist predator of eggs and larvae and an obligate parasitoid on prepupae of *Leptinotarsa* species (Chaboussou, 1939; Weber et al., 2006). The two-spotted stink bug, *Perillus bioculatus* (Fabricius) (Hemiptera: Pentatomidae), prefers *L. decemlineata* in the field (Knight, 1923), and is considered a specialist on exposed chrysomelid larvae (Heimpel, 1991). Like the two generalists, *L. grandis* and *P. bioculatus* are common in

potato fields (Hemenway & Whitcomb, 1967; Heimpel & Hough-Goldstein, 1992; Ferro, 1994).

The aims of this study were to determine the half-lives of DNA detectability for *L. decemlineata* in the guts of this suite of key predators of this pest, to demonstrate the use of these half-lives to weight the incidence of prey in the guts of field-collected predators, and to determine how this weighting affects the ranking of predators as biological control agents in a field situation.

Materials and methods

Field collections

Predators were collected from conventionally tilled potato fields in 2006 and 2007 as part of a larger study on the influence of habitat management on *L. decemlineata* biological control at the Beltsville Agricultural Research Center (BARC) in Beltsville, MD, USA (39°01′N, 76°55′W); complete details of potato management and experimental design are presented in Szendrei et al. (2010). Ten predator samples were taken between 26 May and 25 July in 2006, and eight between 4 June and 30 July in 2007, by hand-searching foliage. Predators were placed immediately in 75% EtOH at 4 °C in individual 5-ml glass vials that were stored until DNA extraction. We did not observe any regurgitation of gut contents into the EtOH.

Laboratory feeding studies

Prey insects. To minimize variability in half-life estimates, we used laboratory-reared animals. *Leptinotarsa decemlineata* originated from animals collected on potatoes at BARC in August 2007. The false potato beetle, *Leptinotarsa juncta* (Germar), originated from animals collected on horsenettle (*Solanum carolinense* L., Solanaceae) in West Virginia pastures in 2003 and potato fields at BARC in 2004. Both colonies were maintained at the Invasive Insect Biocontrol and Behavior Laboratory at BARC, as described previously (Greenstone et al., 2007). *Leptinotarsa juncta* can co-occur in potato fields with *L. decemlineata*, either on potato or on *S. carolinense* on the margins of or within fields (Hemenway & Whitcomb, 1967). We used *L. juncta* and predators fed upon it as negative controls for all assays, and also to sustain normal predator metabolism during the feeding studies. Besides the two *Leptinotarsa* species and four predator species involved in the half-life experiments, we tested the *L. decemlineata* primers against 36 other potential alternate prey species collected from the study locality or known to occur in potato in the Eastern USA (Table 1).

To standardize the quantity of DNA consumed by each predator, we used a single egg of *L. decemlineata*, 24–48 h post-oviposition, as the prey item for half-life determination. Eggs were separated carefully from masses oviposited on potato foliage.

Table 1 Additional species tested for specificity against *Leptinotarsa decemlineata*-specific polymerase chain reaction primers

Species	Collecting locality (all in USA)
ARANEAE	
Lycosidae	
Pardosa milvina (Hentz)	Beltsville, MD
Rabidosa rabida (Walckenaer)	Beltsville, MD
Theridiidae	
Achaearanea tepidariorum (CL Koch)	Ellicott City, MD
INSECTA	
Anthocoridae	
Orius insidiosus (Say)	Charleston, SC
Aphididae	
Macrosiphum euphorbiae (Thomas)	Beltsville, MD
Cerambycidae	
Tetraopes tetrophthalmus (Förster)	Beltsville, MD
Carabidae	
Abacidus permundus (Say)	Beltsville, MD
Agonum striatopunctatum Dejean	Beltsville, MD
Agonum punctiforme (Say)	Champaign, IL
Amara aenea (DeGeer)	Beltsville, MD
Amara anthobia A Villa & GB Villa	Beltsville, MD
Amara familiaris (Duftschmid)	Beltsville, MD
Amara cupreolata Putzeys	Beltsville, MD
Anisodactylus sanctaecrucis (Fabricius)	Champaign, IL
Bembidion affine Say	Beltsville, MD
Bembidion quadrimaculatum oppositum Say	Beltsville, MD
Bradycellus insulsus (Casey)	Beltsville, MD
Elaphropus anceps (LeConte)	Beltsville, MD
Elaphropus xanthopus (Dejean)	Beltsville, MD
Harpalus herbivagus Say	Champaign, IL
Harpalus fulgens Csiki	Beltsville, MD
Harpalus indigens Casey	Champaign, IL
Stenolophus dissimilis Dejean	Beltsville, MD
Stenolophus conjunctus (Say)	Beltsville, MD
Coccinellidae	
Coccinella septempunctata L.	Beltsville, MD
Epilachna varivestis Mulsant	Beltsville, MD
Harmonia axyridis (Pallas)	Beltsville, MD
Lygaeidae	
Geocorus punctipes (Say)	Beltsville, MD
Pentatomidae	
Euschistus servus (Vollenhoven)	Beltsville, MD
Oebalus pugnax (Fabricius)	Beltsville, MD
Thripidae	
Franklinella occidentalis (Pergande)	Charleston, SC

Predatory insects. With the exception of *L. grandis*, all predators used in this research were derived from laboratory colonies, to provide experimental cohorts of known

age, environmental conditions, and dietary history. Establishment and maintenance of the *C. maculata* colony was described previously (Greenstone et al., 2007). The *P. maculiventris* colony originated with adults collected from alfalfa in Boone County, MO, USA, in the summer of 2000, and had been maintained continuously for ca. 120 generations by the protocol of Coudron et al. (2002) at the time of the experiment. The *P. bioculatus* colony originated from adults collected from potato fields at BARC in the summer of 2007, and had been maintained continuously for ca. 15 generations by the protocol of Coudron & Kim (2004); both pentatomids were reared at the Biological Control of Insects Research Laboratory in Columbia, MO, USA, on excess quantities of coddled fourth instars of the cabbage looper, *Trichoplusia ni* (Hübner) (Wittmeyer et al., 2001; Coudron et al., 2002). Early third instars collected within 8 h of molting, or unmated adults collected 5 days after eclosion, were shipped at 6 ± 1 °C (Coudron et al., 2007) from Missouri to Maryland, where all feeding studies were performed. The animals were provided with water but deprived of food during the 48-h shipment; feeding studies were started upon arrival in Beltsville.

Adult *L. grandis* were collected by hand on 8 August 2007 from potato fields at BARC and placed in group cages with water wicks and maintained on *L. juncta* larvae for 3 weeks prior to the feeding experiments.

Feeding protocol. Details of the feeding protocol to determine detectability half-lives for *L. decemlineata* DNA were given previously (Greenstone et al., 2007). Briefly, to ensure that the animals were sufficiently hungry to feed, they were starved before the experiment for 24 h (48 h for *P. maculiventris* and 120 h for *L. grandis*, because they would not feed readily at 24 h), transferred to individual Petri dishes with water wicks, and placed overnight in an incubator programmed to simulate mean hourly temperatures 15 cm above the soil surface and appropriate photoperiod for late May (BARC, 2003; feeding under field conditions is imperative because half-lives are temperature-sensitive (Hagler & Naranjo, 1997; Von Berg et al., 2008). Feeding trials were begun the following day at 10:00 hours. Except for *L. grandis*, which lacks free-living larvae, separate trials were performed for adults and third instars.

Each predator was fed a single *L. decemlineata* egg and observed until it had consumed it; those that had not consumed the egg within 2 h were dropped from the experiment. With the exception of adult *L. grandis*, which had to be hand-collected, and nymphal *P. maculiventris*, because the *Podisus* colony was lost in a laboratory accident before sufficient animals were obtained, 20 individuals of each species were harvested at 4-h intervals beginning with cessation of feeding as time t = 0 and ending at t = 20 h. Preliminary analysis showed that this was insufficient for the two pentatomid species, so additional groups of 20 animals were run at 24-h intervals out to 96 or 120 h. The numbers of *L. grandis* adults and *P. maculiventris* nymphs run at each interval are given in Table 2.

Animals in the t = 0 h groups were frozen at −20 °C immediately after consuming the *L. decemlineata* egg. For those designated for later time points, any remaining *L. decemlineata* egg chorion was removed, 3–5 *L. juncta* eggs were provided, and the animal was returned to the incubator. At its designated time since feeding, each remaining predator was removed from the incubator and frozen at −20 °C until molecular assay.

DNA procedures. DNA was extracted and purified per Greenstone et al. (2005); field-collected animals were removed from the EtOH into which they had been collected, blotted on tissues, and air-dried before extraction. Protocols for preliminary and species-specific PCRs, species-specific PCR primer design, and agarose gel electrophoresis were provided in Greenstone et al. (2007); primer sequences, annealing temperatures, and amplicon sizes are given in Table 3. Each PCR reaction included two positive (single *L. decemlineata* egg) controls, two positive fed-predator (t = 0) controls, two each of three kinds of negative controls – *L. juncta*, *L. juncta*-fed predator, and starved predator – and one no-DNA control. Additionally, control reactions utilizing the same PCR cocktail and plates were run simultaneously with all samples to verify that the DNA in the samples was amplifiable: for the half-life experiments these were the predator-specific reactions; control reactions for the field samples employed generic cytochrome oxidase I primers (Simon et al., 1994).

Statistical analysis. For each species and stage combination, the half-life for DNA detectability and its 95% fiducial limits were determined with the two-parameter probit

Table 2 Numbers of *Podisus maculiventris* nymphs and *Lebia grandis* adults run at each time interval

Interval (h)	No. *P. maculiventris*	No. *L. grandis*
0	20	12
4	20	15
8	17	11
12	20	12
16	13	9
20	20	
48	20	
72	20	

Table 3 Primer sequences

Species	T_a	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)	Size
Prey				
Leptinotarsa decemlineata	54	Cpb5S – CCTTTTCTCTTGGGCAGTTAT	Cpb6A – TTATCCCAAATCCAGGTAGAAT	214
Leptinotarsa juncta	53	FpbWVS1 – CACTGTCATCAAATATTGCCCAT	FpbWVA1 – TGCTAAAACGGGAGGGA	219
Predators				
Coleomegilla maculata	53	Cmac1S – ACAGTTTATCCTCCCTTATCCTCTA	Cmac2B – TTTTTTCTGCTTTCTTGAGTGAAT	414
Lebia grandis	59	Lg3S – AAGACTAGTTGAAAGAGGAGCAGGTA	Lg3A – TCCAACAGATCAAACAAATAAAGGTA	223
Perillus bioculatus	60	Per1S – ATG CGC CCA ATA GGA AT	Per1A – TTG ATA ATA CAT AGT GGA AGT GG	633
Podisus maculiventris	55	Pod2S – GATCAGTTGGAATTACCGCTTTAT	Pod2A – TTGGATTTTTAGTCACTTAGGGTTTA	683

T_a, annealing temperature (°C); size, size of amplicon in bp.

model (Proc PROBIT; SAS Institute, 1999). Species-stage combinations whose 84% fiducial limits do not overlap are statistically different at $P<0.05$ (Payton et al., 2003).

Half-life adjustment of Leptinotarsa decemlineata *incidence in the gut.* For each species-stage combination, the proportion positive for *L. decemlineata* DNA of field-collected animals was substituted into the probit regression equation, derived from the laboratory feeding study for that species and stage. The regression was then solved to obtain the time since feeding, i.e., the value of the explanatory variable required to obtain that observed percentage. This quantity was then substituted into the probit regression equation as the explanatory variable for that species-stage combination displaying the middle-most half-life among all of the species-stage combinations to arrive at an adjusted observed proportion. In effect, we ask what proportion positive each species-stage would exhibit if their half-lives all equaled that of the middle-most species-stage combination. Choice of the middle-most half-life was arbitrary: any species-stage combination could be used as the group to which adjustments were made and produce the same rankings.

Results

The *L. decemlineata* primers exhibited perfect specificity, amplifying DNA of the target species but none of other species in the experiment or potentially present in the field. *Leptinotarsa decemlineata* eggs and/or larvae were present in the field on all sampling dates (Szendrei et al., 2010). Of 351 predators collected from the field, 46% had detectable *L. decemlineata* DNA in the gut, with the incidence ranging from 11% in *C. maculata* adults to 95% in *P. bioculatus* nymphs (Table 4). DNA digestive rates varied greatly, with

Table 4 Molecular gut-content analysis of field collected predators, with 2006 and 2007 samples combined. Numbers are individual predators hand-collected from conventionally tilled potato plots at the Beltsville Agricultural Research Center. Positives are individuals positive by polymerase chain reaction for *Leptinotarsa decemlineata* cytochrome oxidase I DNA

Species	Life stage	Total	Positives	Proportion
Coleomegilla maculata	Adult	141	15	0.11
	Larva	2	0	0
Lebia grandis	Adult	56	18	0.32
Perillus bioculatus	Adult	58	49	0.85
	Nymph	41	39	0.95
Podisus maculiventris	Adult	44	33	0.75
	Nymph	9	7	0.78
Total		351	161	0.46

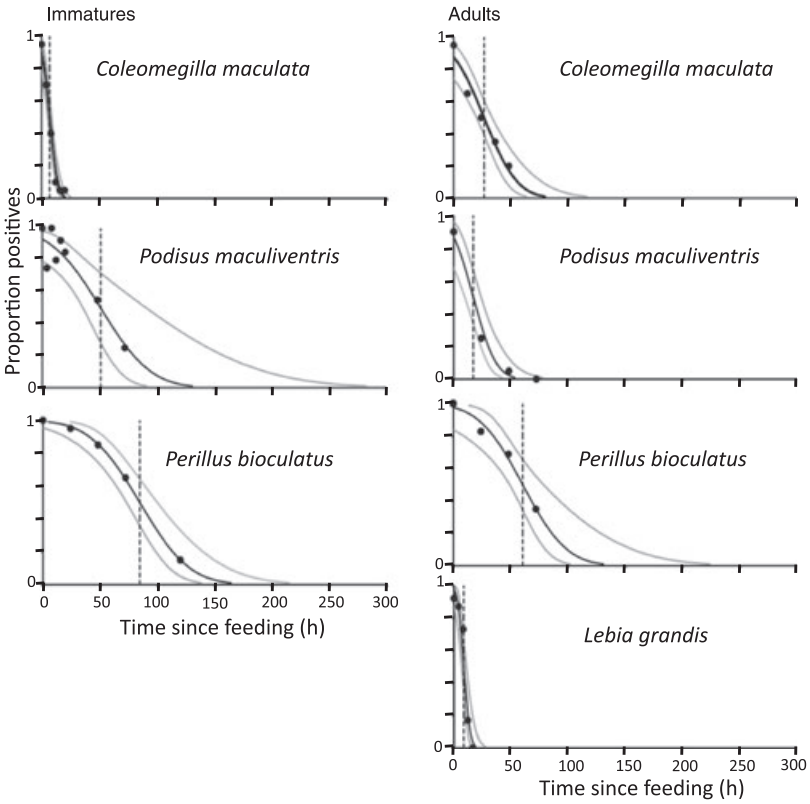

Figure 1 Results of *Leptinotarsa decemlineata* DNA half-life studies for immature and adult predators. Regressions and 95% fiducial limits were fitted by a two-parameter probit model (Proc PROBIT; SAS Institute, 1999). The dotted vertical line indicates the half-life.

Table 5 Results of half-life analysis for detectability by polymerase chain reaction of cytochrome oxidase I DNA consumed from a single egg of *Leptinotarsa decemlineata* in predators held under simulated field conditions

Species/stage	Half-life (h)	95% fiducial limits	Intercept	Slope
Immature				
Coleomegilla maculata	7.0e	5.2–8.6	1.2776	−0.1820
Lebia grandis	—			
Perillus bioculatus	84.4a	72.8–99.7	2.4583	−0.0291
Podisus maculiventris	50.9b	35.5–90.8	1.4824	−0.0291
Adult				
Coleomegilla maculata	26.4c	19.9–33.3	1.1618	−0.0440
Lebia grandis	8.6e	6.7–10.7	1.9453	−0.2274
Perillus bioculatus	60.5b	49.1–78.6	2.0693	−0.0332
Podisus maculiventris	17.1d	11.8–22.7	1.0276	−0.0581

Half-lives were determined with a two-parameter probit model (Proc PROBIT; SAS Institute, 1999). Half-lives followed by different letters are statistically different (Payton et al., 2003). Immature. *C. maculata* and *P. maculiventris* half-lives from Greenstone et al. (2007), by permission.

the pentatomids tending to be slower (Figure 1). The half-lives of the predators and their stages ranged from 7.0 h in larval *C. maculata* to 84.4 h in nymphal *P. bioculatus* (Table 5), indicating that the probability of collecting a *P. bioculatus* nymph containing detectable *L. decemlineata* cytochrome oxidase I DNA is greater than that for a *C. maculata* larva with identical feeding history. The half-lives of adults and immatures within each species were statistically different; half-lives of all adults were also statistically different from one another, as were those of all immatures (Table 5). The data fit the probit model well, with $P<0.001$ in all cases. The raw incidences were adjusted as described above by indexing them to that of *P. maculiventris* nymphs, which was the species-stage combination whose detectability half-life was nearest the mean half-life of all species and stages studied (Table 6).

When the raw incidence values are used, the members of the predator complex rank thus: *C. maculata* adults < *L. grandis* adults < *P. maculiventris* adults < *P. maculiventris* nymphs < *P. bioculatus* adults < *P. bioculatus* nymphs (no target DNA was detected in *C. maculata* larvae). Adjusted incidences change the rankings for the members of the same complex as follows: *C. maculata*

Table 6 Incidence (%) of cytochrome oxidase I DNA detected by polymerase chain reaction assay in predators collected from conventionally tilled potato plots at the Beltsville Agricultural Research Center in 2006 and 2007 (combined). For each species, the raw incidence was used to calculate the corresponding time since feeding from its probit model, which was then entered into the probit model for *Podisus maculiventris* nymphs to calculate the adjusted incidence

		Incidence (%)	
Predator	Life stage	Raw	Adjusted
Coleomegilla maculata	Adult	10.6	39.4
	Larva	0	0
Lebia grandis	Adult	32.1	88.0
Perillus bioculatus	Adult	84.5	73.0
	Nymph	95.1	75.2
Podisus maculiventris	Adults	75.0	90.4
	Nymph	77.8	77.8

adults < *P. bioculatus* adults < *P. bioculatus* nymphs < *P. maculiventris* nymphs < *L. grandis* adults < *P. maculiventris* adults. The adjusted rankings place adults of the polyphagous pentatomid *P. maculiventris* first, ahead of the stenophagous carabid *L. grandis*. This is a dramatic change from the original rankings based on raw incidence (Table 4), which placed all stages of the two pentatomids first, with the stenophagous *P. bioculatus* before the polyphagous *P. maculiventris*.

Discussion

Molecular gut-content assay has revolutionized our ability to determine the role of predators in suppressing insect pest populations. Arranging the members of a predator complex by incidence of a pest in the gut is a reasonable first step in ranking them for conservation biological control (Birkhofer et al., 2008; Fournier et al., 2008). For field-collected predators of *L. decemlineata*, ranking by raw *L. decemlineata* DNA incidence places both stages of *P. bioculatus* first, followed by both stages of *P. maculiventris*, adults of *L. grandis*, and adults of *C. maculata*. However, when the incidence is adjusted for predator-specific differences in prey detectability half life, *P. maculiventris* adults move into first place, followed by *L. grandis* adults, *P. maculiventris* nymphs, both stages of *P. bioculatus*, and adults of *C. maculata*. The most striking change is the elevation of *L. grandis* from next-to-worst to next-to-best candidate. It is also notable that the adjusted values rank as the two best candidates a polyphagous hemipteran and a stenophagous coleopteran, suggesting that degree of pest species-specificity may not be the best predictor for the effectiveness of a natural enemy.

Our half-life adjustment does not account for differences in feeding pattern amongst predator species over time of day, nor for repeat feeding on the same prey before a prior prey item becomes essentially undetectable. If, for example, *P. maculiventris* nymphs are apt to eat another egg before the first becomes undetectable, its detectability half-life will appear lengthened (Naranjo & Hagler, 1998). The impact of feeding interval on the adjusted incidence cannot be determined without knowing the feeding frequency of all predators in the complex. The attack rate of adult female *P. maculiventris* on *L. decemlineata* larvae but not eggs has been measured in the field, giving a feeding interval of roughly 44 h (after O'Neil, 1997). However, attack rates have not been measured in the field for the other members of this predator complex. Finally, consumption of alternate prey species would increase feeding intervals for *L. decemlineata* meals, an effect that would be smaller for the more stenophagous species, *L. grandis* and *P. bioculatus*, than for those with broader diets.

There are several outstanding technical issues in the application of molecular gut-content assay data to decision-making in biological control. One is determining whether prey remains entered the gut through predation or via one or more alternative food-chain pathways. Molecular gut content assays may detect scavenging or secondary predation, making it necessary to estimate the likelihood of such events for each system under study. The probability of scavenging will depend upon each predator's innate preference for live vs. dead prey, availability of cadavers, and their state of decay upon encounter by the predator (Foltan et al., 2005). It might be possible to detect the presence or absence of enzymes found in living animals that are rapidly degraded in cadavers, but this will require fundamental research to discover which enzymes behave in this fashion (Juen & Traugott, 2005). Whether or not secondary predation is likely to be an issue depends on the system under study and the sensitivity of the assay (Harwood et al., 2001; Sheppard et al., 2005).

The quantity of prey protein or DNA in the gut can be estimated by ELISA or quantitative PCR. This quantity may decay with time (Fichter & Stephen, 1981; Sopp & Sunderland, 1987; Harwood et al., 2001; Fournier et al., 2006; Weber & Lundgren, 2009), enabling estimation of a half-life for prey remains (Symondson & Liddell, 1995). This is not, however, the same as the detectability half-life: the latter's application to gut-content data analysis provides a predator-species-specific standard of comparison for the value of a positive by removing the bias that would otherwise overvalue the role of species with longer detectability periods (Sopp et al., 1992; Chen et al., 2000; Fournier et al., 2006; Hosseini et al., 2008).

The data from standard molecular gut-content assays, whether serological or DNA-based, are positives and negatives. The positives, however, subsume unknown variables that affect the detectability of the target molecule. These include the number and size of prey eaten, the stage of prey eaten, the time since consuming a prey item, the temperature regime from the time of consumption until the animal was collected, and whether alternate prey were consumed during the feeding interval (Sopp & Wratten, 1986; Sopp et al., 1992; Hagler & Naranjo, 1997; Weber & Lundgren, 2009). The largest component of prey size is prey stage, which may be marked by stage-specific proteins (Goodman et al., 1997; Fournier et al., 2006); this has made it possible to identify prey stage-specific predation in the field by immunoassay (Sigsgaard et al., 2002; Fournier et al., 2008). DNA-based assays cannot distinguish stages. Reverse-transcriptase (RT-) PCR could do so, but this would require a major research effort to identify stage-specific proteins and the RNA sequences encoding them. Provided the stage consumed can be determined, the number of individuals consumed might be estimated by RT-PCR (Nejtstgaard et al., 2007; Zhang et al., 2007; Weber & Lundgren, 2009). Alternatively, the mean number of prey items in the gut represented by a sample of gut-content assay positives could be estimated by applying the Poisson distribution (Nakamura & Nakamura, 1977; Greenstone, 1979; Lister et al., 1987), but this alone would not translate to a rate of feeding without knowledge of the feeding interval and size of the prey item.

Finally, by making use of the observation that shorter DNA fragments tend to become undetectable more slowly than longer ones (Agustí et al., 1999; Zaidi et al., 1999; Chen et al., 2000). Hoogendoorn & Heimpel (2001) showed that one may determine the time since feeding by using several sets of PCR primer pairs that target fragments of different lengths. In at least one case, however, this relationship between amplicon size and digestive rate did not obtain (Juen & Traugott, 2005), suggesting that this approach may be taxon- or temperature-dependent. Combining all of these factors into a true quantitative, i.e., per capita, estimate of predator impact on a pest will require a significant modeling effort; the success of previous attempts to model various aspects of predation from gut-content data provides grounds for optimism (Sopp et al., 1992; Naranjo & Hagler, 2001).

To study multiple predator effects empirically, one should not disturb the real-life interactions that characterize a diverse species community. Molecular gut-content analysis enables assessment of feeding by an entire predator complex in its unmanipulated environment. Given a sufficiently robust model for deriving quantitative estimates of feeding rate, gut content analysis can support estimates of the contribution of each predator in a complex to the suppression of the pest, and thereby identify those species and stages most worthy of conservation. For example, the 'sampling effect' (Tilman, 1999; Loreau et al., 2001), where a dominant predator affects prey abundance, may be revealed by molecular gut content analysis. In our example, half-life adjustment of the raw incidence of *L. decemlineata* DNA in the gut revealed *P. maculiventris* and *L. grandis* to be more effective biological control agents of *L. decemlineata*, on a per capita basis, than the other species in the complex, suggesting that conservation management practices should target them.

We used a realistic but simple predator–prey system to develop these ideas. By using multiple DNA primers for predators and prey present in more complex systems, entire networks of intraguild predators and alternate prey can be mapped (Agustí et al., 2003; Harwood et al., 2007; Saccaggi et al., 2008). The results would provide additional insight into the mechanisms of biological control, and aid managers tasked with modifying cultural methods to enhance biocontrol of pests. The cost-effectiveness and relative ubiquity of molecular laboratories allows for the quick development of PCR primers for prey and predators; primers for cosmopolitan predator–prey systems can also be shared among laboratories, further facilitating the use of these methods (Symondson, 2002; Harwood & Greenstone, 2008).

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References

Agustí N, de Vicente MC & Gabarra R (1999) Development of sequence amplified characterized region (SCAR) markers of *Helicoverpa armigera*: a new polymerase chain reaction-based technique for predator gut analysis. Molecular Ecology 8: 1467–1474.

Agustí N, Shayler P, Harwood JD, Vaughan IP, Sunderland KD & Symondson WOC (2003) Collembola as alternative prey sustaining spiders in arable ecosystems: prey detection within predators using molecular markers. Molecular Ecology 12: 3467–3475.

Andow DA & Risch S (1985) Predation in diversified agroecosystems: relations between a coccinellid predator *Coleomegilla maculata* and its food. Journal of Applied Ecology 22: 357–372.

BARC (2003) Beltsville Area Research Center, Station #2 North Farm – History and Information. Available at: http://www.ars.usda.gov/Services/docs.htm?docid= 6443. Last accessed on 13 May 2010.

Benton AH & Crump J (1981) Observations on the spring and summer behavior of the 12-spotted ladybird beetle, *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae). Journal of the New York Entomological Society 89: 102–105.

Birkhofer B, Gavish-Regev E, Endlweber K, Lubin YD, Von Berg K et al. (2008) Cursorial spiders retard initial aphid population growth at low densities in winter wheat. Bulletin of Entomological Research 98: 249–255.

Calder CR, Harwood JD & Symondson WOCS (2005) Detection of scavenged material in the guts of predators using monoclonal antibodies: a significant source of error in measurement of predation? Bulletin of Entomological Research 95: 1–6.

Cardinale BJ, Harvey C, Gross K & Ives AR (2003) Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. Ecology Letters 6: 857–865.

Chaboussou F (1939) Contribution à l'étude biologique de *Lebia grandis* Hentz, prédateur américain du Doryphore. Annales Épiphyties et de Phytogénetetique 5: 387–433.

Chang GC & Kareiva P (1999) The case for indigenous generalists in biological control. Theoretical Approaches to Biological Control (ed. by BA Hawkins & HV Cornell), pp. 103–115. Cambridge University Press, Cambridge, UK.

Chen YK, Giles KL, Payton ME & Greenstone MH (2000) Identifying key cereal aphid predators by molecular gut analysis. Molecular Ecology 9: 1887–1898.

Coudron TA & Kim Y (2004) Life history and cost analysis for continuous rearing of *Perillus bioculatus* (F.) (Heteroptera: Pentatomidae) on a zoophytophagous artificial diet. Journal of Economic Entomology 97: 807–812.

Coudron TA, Wittmeyer JL & Kim Y (2002) Life history and cost analysis for continuous rearing of *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) on a zoophytophagous diet. Journal of Economic Entomology 95: 1159–1168.

Coudron TA, Ellersieck MR & Shelby KS (2007) Influence of diet on long-term cold storage of the predator *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae). Biological Control 42: 186–195.

Ferro DN (1994) Biological control of the Colorado potato beetle. Advances in Potato Pest Biology and Management (ed. by GW Zehnder, ML Powelson, RK Jansson & KVA Raman), pp. 357–375. American Phytopathological Society Press, St. Paul, MN, USA.

Fichter BL & Stephen WP (1981) Time related decay in prey antigens ingested by the predator *Podisus maculiventris* (Hemiptera, Pentatomidae) as detected by ELISA. Entomolgia Experimentalis et Applicata 51: 404–407.

Foltan P, Sheppard S, Konvicka M & Symondson WOC (2005) The significance of facultative scavenging in generalist predator nutrition: detecting decayed prey in the guts of predators using PCR. Molecular Ecology 14: 4147–4158.

Fournier V, Hagler JR, Daane KM, de León JH, Groves RL et al. (2006) Development and application of a glassy-winged and smoke-tree sharpshooter egg-specific predator gut-content ELISA. Biological Control 37: 108–111.

Fournier V, Hagler JR, Daane K, de León J & Groves R (2008) Identifying the predator complex of *Homalodisca vitripennis* (Hemiptera: Cicadellidae): a comparative study of the efficacy of an ELISA and PCR gut content assay. Oecologia 157: 629–640.

Gariepy TD, Kuhlman U, Gillot C & Erlandson M (2007) Parasitoids, predators, and PCR: the use of diagnostic molecular markers in biological control of arthropods. Journal of Applied Entomology 131: 225–240.

Goodman CL, Greenstone MH & Stuart MK (1997) Monoclonal antibodies to vitellins of bollworm and tobacco budworm (Lepidoptera: Noctuidae): biochemical and ecological implications. Annals of the Entomological Society of America 90: 83–90.

Greenstone MH (1979) Spider feeding behaviour optimises dietary essential amino acid composition. Nature 181: 501–503.

Greenstone MH (1996) Serological analysis of arthropod predation: past, present and future. The Ecology of Agricultural Pests – Biochemical Approaches (ed. by WOC Symondson & JE Liddell), pp. 265–300. Chapman and Hall, London, UK.

Greenstone MH & Dickens JC (2005) The production and appropriation of chemical signals among plants, herbivores, and predators. Ecology of Predator–Prey Interactions (ed. by P Barbosa), pp. 139–165. Oxford University Press, Oxford, UK.

Greenstone MH & Hunt JH (1993) Determination of prey antigen half-life in *Polistes metricus* using a monoclonal antibody-based immunodot assay. Entomologia Experimentalis et Applicata 68: 1–7.

Greenstone MH & Morgan CE (1989) Predation on *Heliothis zea* (Lepidoptera: Noctuidae): an instar-specific ELISA for stomach analysis. Annals of the Entomological Society of America 82: 45–49.

Greenstone MH & Sunderland KD (1999) Why a symposium on spiders in agroecosystems now? Journal of Arachnology 27: 267–269.

Greenstone MH, Rowley DL, Heimbach U, Lundgren JG, Pfannenstiel RS & Rehner SA (2005) Barcoding generalist predators by polymerase chain reaction: carabids and spiders. Molecular Ecology 14: 3247–3266.

Greenstone MH, Rowley DL, Weber DC, Payton ME & Hawthorne DJ (2007) Feeding mode and prey detectability half-lives in molecular gut-content analysis: an example with two

predators of the Colorado potato beetle. Bulletin of Entomological Research 97: 201–209.

Groden E, Drummond FA, Casagrande RA & Haynes DL (1990) *Coleomegilla maculata* (Coleoptera: Coccinellidae): its predation upon the Colorado potato beetle (Coleoptera: Chrysomelidae) and its incidence in potatoes and surrounding crops. Journal of Economic Entomology 83: 1306–1315.

Gurr GM, Wratten SD & Barbosa P (2000) Success in conservation biological control of arthropods. Biological Control: Measures of Success (ed. by G Gurr & S Wratten), pp. 105–132. Kluwer Academic Publishers, Dordecht, The Netherlands.

Hagler JR & Naranjo SE (1997) Measuring the sensitivity of an indirect predator gut content ELISA: detectability of prey remains in relation to predator species, temperature, time, and meal size. Biological Control 9: 112–119.

Harwood JD & Greenstone MH (2008) Molecular diagnosis of natural enemy–host interactions. Recent Advances in Insect Physiology, Toxicology and Molecular Biology (ed. by N Liu), pp. 41–57. Research Signpost, Kerala, India.

Harwood JD, Phillips SW, Sunderland JD & Symondson WOC (2001) Secondary predation: quantification of food chain errors in an aphid-spider-carabid system using monoclonal antibodies. Molecular Ecology 10: 2049–2057.

Harwood JD, Sunderland KD & Symondson WOC (2004) Prey selection by linyphiid spiders: molecular tracking of the effects of alternative prey on rates of aphid consumption in the field. Molecular Ecology 13: 3549–3560.

Harwood JD, Desneux N, Yoo HJS, Rowley DL, Greenstone MH et al. (2007) Tracking the role of alternative prey in soybean aphid predation by *Orius insidiosus*: a molecular approach. Molecular Ecology 16: 4390–4400.

Hazzard RV, Ferro DN, van Driesche RG & Tuttle AF (1991) Mortality of eggs of the Colorado potato beetle (Coleoptera: Chrysomelidae) from predation by *Coleomegilla maculata* (Coleoptera: Coccinellidae). Environmental Entomology 20: 841–848.

Heimpel GE (1991) Searching Behavior and Functional Response of *Perillus bioculatus*, a Predator of the Colorado Potato Beetle. MS Thesis, University of Delaware, Newark, DE, USA.

Heimpel GE & Hough-Goldstein JA (1992) A survey of arthropod predators of *Leptinotarsa decemlineata* (Say) in Delaware potato fields. Journal of Agricultural Entomology 9: 137–142.

Hemenway R & Whitcomb WH (1967) Ground beetles of the genus *Lebia* Latreille in Arkansas (Coleoptera: Carabidae): ecology and geographical distribution. Arkansas Academy of Science Proceedings 21: 15–20.

Hilbeck A, Eckel C & Kennedy GG (1997) Predation on Colorado potato beetle eggs by generalist predators in research and commercial potato plantings. Biological Control 8: 191–196.

Hoogendoorn M & Heimpel GE (2001) PCR-based gut content analysis of insect predators: using ribosomal ITS-1 fragments from prey to estimate predation frequency. Molecular Ecology 10: 2059–2067.

Hosseini R, Schmidt O & Keller MA (2008) Factors affecting detectability of prey DNA in the gut of invertebrate predators: a polymerase chain reaction-based method. Entomologia Experimentalis et Applicata 126: 194–202.

Hough-Goldstein J & McPherson D (1996) Comparison of *Perillus bioculatus* and *Podisus maculiventris* (Hemiptera: Pentatomidae) as potential control agents of the Colorado potato beetle (Coleoptera: Chrysomelidae). Journal of Economic Entomology 89: 1116–1123.

Ives AR, Cardinale BJ & Snyder WE (2005) A synthesis of subdisciplines: predator–prey interactions, and biodiversity and ecosystem functioning. Ecology Letters 8: 102–116.

Juen A & Traugott M (2005) Detecting predation and scavenging by DNA gut-content analysis: a case study using a soil insect predator–prey system. Oecologia 142: 344–352.

Knight HH (1923) Studies of the life history and biology of *Perillus bioculatus* Fabricius, including observations on the nature of the color pattern. 19th Report of the State Entomologist of Minnesota, Minnesota Agricultural Experiment Station, St Paul, MN, USA (1923): 50–96.

Landis DA, Wratten SD & Gurr GM (2000) Habitat management to conserve natural enemies of arthropod pests. Annual Review of Entomology 45: 175–201.

Lister A, Usher MB & Block W (1987) Description and quantification of field attack rates by predatory mites: an example using an electrophoresis method with a species of Antarctic mite. Oecologia 72: 185–191.

Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP et al. (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. Science 294: 804–808.

Lundgren JG (2009) Relationships of Natural Enemies and Non-Prey Foods. Springer, London, UK.

Ma J, Li D, Keller M, Schmidt O & Feng X (2005) A DNA marker to identify predation of *Plutella xylostella* (Lep., Plutellidae) by *Nabis kinbergii* (Hem., Nabidae) and *Lycosa* sp. (Araneae, Lycosidae). Journal of Applied Entomology 129: 330–335.

Nakamura M & Nakamura K (1977) Population dynamics of the chestnut gall wasp, *Dryocosmus kuriphilus* Yamatsu (Hymenoptera: Cynipidae). V. Estimation of the effect of predation by spiders on the mortality of imaginal wasps based on the precipitin test. Oecologia 27: 97–116.

Naranjo SE & Hagler JR (1998) Characterizing and estimating the impact of heteropteran predation. Predatory Heteroptera: Their Ecology and Use in Biological Control (ed. by M Coll & J Ruberson), pp. 170–197. Thomas Say Symposium Proceedings, Entomological Society of America, Lanham, MD, USA.

Naranjo SE & Hagler JR (2001) Toward the quantification of predation with predator gut immunoassays: a new approach integrating functional response behavior. Biological Control 20: 175–189.

Nejtstgaard JC, Fisscher ME, Simonelli P, Troedsson C, Brakel M et al. (2007) Quantitative PCR to estimate copepod feeding. Marine Biology 153: 565–577.

O'Neil RJ (1997) Functional response and search strategy of *Podisus maculiventris* (Heteroptera: Pentatomidae) attacking Colorado potato beetle (Coleoptera: Chrysomelidae). Environmental Entomology 26: 1183–1190.

Otto SB, Berlow EL, Rank NE, Smiley J & Brose U (2008) Predator diversity and identity drive interaction strength and trophic cascades in a food web. Ecology 89: 134–144.

Payton ME, Greenstone MH & Schenker N (2003) Overlapping confidence intervals or standard error intervals: what do they mean in terms of statistical significance? Journal of Insect Science 3: 1–6.

Rabb RL, Stinner RE & van den Bosch R (1976) Conservation and augmentation of natural enemies. Theory and Practice of Biological Control (ed. by CB Huffaker & PS Messenger), pp. 233–254. Academic Press, New York, NY, USA.

Root RB (1973) Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). Ecological Monographs 43: 95–124.

Saccaggi DL, Krüger K & Pietersen G (2008) A multiplex PCR assay for the simultaneous identification of three mealybug species (Hemiptera: Pseudococcidae). Bulletin of Entomological Research 98: 27–33.

SAS Institute (1999) SAS/STAT User's Manual, Ver 8, 4th edn. SAS Institute, Cary, NC, USA.

Sheppard SK & Harwood JD (2005) Advances in molecular ecology: tracking trophic links through predator-prey food webs. Functional Ecology 19: 751–762.

Sheppard SK, Bell J, Sunderland KD, Fenlon J, Skervin D & Symondson WOC (2005) Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. Molecular Ecology 14: 4461–4468.

Sigsgaard L, Greenstone MH & Duffield SJ (2002) Egg cannibalism in *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) on sorghum and pigeonpea. BioControl 47: 151–165.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H & Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87: 651–701.

Snyder WE, Chang GC & Prasad RP (2004) Conservation biological control: biodiversity influences the effectiveness of predators. Ecology of Predator–Prey Interactions (ed. by P Barbosa & I Castellanos), pp. 324–343. Oxford University Press, Oxford, UK.

Sopp PI & Sunderland KD (1987) Some factors affecting the detection period of aphid remains in predators using ELISA. Entomologia Experimentalis et Applicata 51: 11–20.

Sopp PI & Wratten SD (1986) Rates of consumption of ceral aphids by some polyphagous predators in the laboratory. Entomologia Experimentalis et Applicata 41: 69–73.

Sopp PI, Sunderland KD, Fenlon JS & Wratten SD (1992) An improved quantitative method for estimating invertebrate predation in the field using an enzyme-linked immunosorbent assay (ELISA). Journal of Applied Ecology 79: 295–302.

Straub CS & Snyder WE (2006) Species identity dominates the relationship between predator biodiversity and herbivore suppression. Ecology 82: 277–282.

Straub CS & Snyder WE (2008) Increasing enemy biodiversity strengthens herbivore suppression on two plant species. Ecology 89: 1605–1615.

Sunderland KD (1988) Quantitative methods for detecting invertebrate predation in the field. Annals of Applied Biology 112: 201–224.

Sunderland KD (1996) Progress in quantifying predation using antibody techniques. The Ecology of Agricultural Pests – Biochemical Approaches (ed. by WOC Symondson & JE Liddell), pp. 419–455. Chapman and Hall, London, UK.

Sunderland KD (1999) Mechanisms underlying the effects of spiders on pest populations. Journal of Arachnology 27: 308–316.

Symondson WOC (2002) Molecular identification of prey in predator diets. Molecular Ecology 11: 627–641.

Symondson WOC & Liddell JE (1995) Decay rates for slug antigens within the carabid predator *Pterostichus melanarius* monitored with a monoclonal antibody. Entomologia Experimentalis et Applicata 75: 245–250.

Symondson WOC, Sunderland KD & Greenstone MH (2002) Can generalist predators be effective biocontrol agents? Annual Review of Entomology 47: 561–594.

Szendrei Z, Greenstone MH, Payton ME & Weber DC (2010) Molecular gut analysis of a predator assemblage reveals the effect of habitat manipulation on biological control in the field. Basic and Applied Ecology 11: 153–161.

Tilman D (1999) The ecological consequences of changes in biodiversity: a search for general principles. Ecology 80: 1455–1474.

Tipping PW, Holko C, Abdul-Baki AA & Aldrich JR (1999) Evaluating *Edovum puttleri* Grissell and *Podisus maculiventris* (Say) for augmentative biological control of Colorado potato beetle in tomatoes. Biological Control 16: 35–42.

Von Berg K, Traugott M, Symondson WOC & Scheu S (2008) The effects of temperature on detection of prey DNA in two species of carabid beetle. Bulletin of Entomological Research 98: 263–269.

Weber DC & Lundgren JG (2009) Detection of predation using qPCR: effect of prey quantity, elapsed time, chaser diet, and sample preservation on detectable quantity of prey DNA. Journal of Insect Science 9: 41.

Weber DC, Rowley DL, Greenstone MH & Athanas MM (2006) Prey preference and prey suitability of the predatory and parasitic carabid beetle, *Lebia grandis*, for several species of *Leptinotarsa* beetles. Journal of Insect Science 6: 09.

Wittmeyer JL, Coudron TA & Adams TS (2001) Ovarian development, fertility and fecundity in *Podisus maculiventris* Say (Heteroptera: Pentatomidae): an analysis of the impact of nymphal, adult, male and female nutritional source on reproduction. Invertebrate Reproduction and Development 39: 9–20.

Zaidi RH, Jaal Z, Hawkes NJ, Hemingway J & Symondson WOC (1999) Can the detection of prey DNA amongst the gut contents of invertebrate predators provide a new technique for quantifying predation in the field? Molecular Ecology 8: 2081–2088.

Zhang GL, Lu ZC, Wan FH & Lövei GL (2007) Real-time PCR quantification of *Bemisia tabaci* (Homoptera: Aleyrodidae) B-biotype remains in predator guts. Molecular Ecology Notes 7: 947–954.