

1 **Title:** Two common invertebrate predators show varying responses to different types of  
2 sentinel prey in a mesocosm study

3

#### 4 **Authors**

5 Arran Greenop.<sup>1,2</sup>, Andreas Cecelja., Ben A Woodcock<sup>1</sup>, Andrew Wilby<sup>2</sup>, Samantha M Cook<sup>3</sup>  
6 & Richard F Pywell<sup>1</sup>.

7 <sup>1</sup> NERC Centre for Ecology & Hydrology, Maclean Building, Crowmarsh Gifford, Wallingford,  
8 Oxfordshire OX10 8BB, UK. Tel. +44(0)1491692415

9 <sup>2</sup> Lancaster Environment Centre, Library Avenue, Lancaster University, Lancaster LA1 4YQ

10 <sup>3</sup> Biointeractions and Crop Protection Department, Rothamsted Research, Harpenden, Herts,  
11 AL5 2JQ, UK.

12

13 **Contact Author:** Arran Greenop, e-mail:arrgre@ceh.ac.uk, 07538808379

14

#### 15 **Acknowledgements**

16 This study was supported by the Centre for Ecology & Hydrology, Rothamsted Research  
17 and Lancaster University. The research was also supported by the Natural Environment  
18 Research Council (NERC) and the Biotechnology and Biological Sciences Research Council  
19 (BBSRC) under research programme NE/N018125/1LTS-M ASSIST – Achieving  
20 Sustainable Agricultural Systems [www.assist.ceh.ac.uk](http://www.assist.ceh.ac.uk).

21

22

23

24

25

26

## 27 Abstract

28 Sentinel prey (an artificially manipulated patch of prey) are widely used to assess the level of  
29 predation provided by natural enemies in agricultural systems. While a number of different  
30 methodologies are currently in use, little is known about how arthropod predators respond to  
31 artificially-manipulated sentinel prey in comparison to predation on free-living prey  
32 populations. We assessed how attack rates on immobilised (aphids stuck to cards) and  
33 artificial (plasticine lepidopteran larvae mimics) sentinel prey differed to predation on free  
34 moving live prey (aphids). Predation was assessed in response to density of the common  
35 invertebrate predators, a foliar active ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae),  
36 and a ground active beetle *Pterostichus madidus* (Coleoptera: Carabidae). Significant  
37 increases in attack rates were found for the immobilised and artificial prey between the low  
38 and high predator density treatments. However, an increased predator density did not  
39 significantly reduce numbers of free living live aphids included in the mesocosms in addition  
40 to the alternate prey. We also found no signs of predation on the artificial prey by the  
41 predator *H. axyridis*. These findings suggest that if our assessment of predation had been  
42 based solely on the foliar artificial prey then no increase in predation would have been found  
43 in response to increased predator density. Our results demonstrate that predators  
44 differentially respond to sentinel prey items which could affect the level of predation recorded  
45 where target pest species are not being used.

46

47 **Key words:** ecosystem services, biological control, artificial caterpillars, aphids, ladybirds,  
48 ground beetle

49

50

51

## 52 Introduction

53 Biodiversity and ecosystem functioning studies are central to understanding how humans  
54 can manage the natural environment to maximise ecosystem services including pollination  
55 and pest control (Bianchi et al., 2006; Cardinale et al., 2006; Foster et al., 2011). Of these  
56 ecosystem services, pest regulation has received considerable attention, much of it relating  
57 to the potential of natural enemies to reduce crop pest populations (Snyder et al., 2008;  
58 Gardiner et al., 2009; Rusch et al., 2016; Begg et al., 2017; Greenop et al., 2018).

59

60 Fundamental to understanding the value of natural pest control in agricultural ecosystems is  
61 an accurate measure of service delivery (Macfadyen et al., 2015). Several methods exist to  
62 assess the suitability and function of pest control provided by natural enemies, ranging from  
63 carefully-selected species assemblages in mesocosm studies conducted under laboratory  
64 conditions (Straub & Snyder, 2006; Northfield et al., 2010) to the exclusion of entire  
65 functional groups under real-world agricultural conditions (Gardiner et al., 2009; Holland et  
66 al., 2012; Woodcock et al., 2016; Mansion-Vaquié et al 2017). The current body of literature  
67 is dominated by studies that either use natural enemy abundances as a proxy for pest  
68 control (Elliott et al. 1999; Schmidt et al. 2005; Bianchi, Booij & Tscharrntke 2006) or infer  
69 predation rates based on pest abundances (Chaplin-Kramer et al. 2011). However, both  
70 approaches have associated problems that may result in the misrepresentation of the true  
71 levels of pest control. For example, inferring predation based on natural enemy abundances  
72 provides no direct measure of prey suppression (Macfadyen et al., 2015). Additionally pest  
73 abundances are often patchy in distribution (Ferguson & Stiling 1996; Winder, Perry &  
74 Holland 1999; Wan *et al.* 2018) and are influenced by bottom up as well as top-down factors  
75 (Chaplin-Kramer et al., 2011). Ultimately, over- or under-estimating the efficacy of natural  
76 pest control limits our capacity to manage and enhance this service to support sustainable  
77 intensification of agricultural systems (Macfadyen et al., 2015; Zalucki et al., 2015).

78

79 To address this issue the use of sentinel prey has become a widely used methodology to  
80 infer rates of natural pest control, particularly for agro-ecosystems (Lövei & Ferrante 2017).  
81 These approaches use an artificially manipulated patch of prey that can be directly  
82 monitored to assess rates of predation under field conditions (Howe et al., 2009; Winqvist et  
83 al., 2011; Roslin et al., 2017). As such they provide a quantitative measure of the number of  
84 prey consumed or parasitized, which is comparable between experimental treatments  
85 (Birkhofer et al., 2017; Lövei & Ferrante, 2017). Two of the most common types of sentinel  
86 prey currently used are: 1) live prey that have been immobilised, either by attaching them to  
87 sticky labels (Winqvist et al., 2011), cards (Bianchi et al., 2005) or tethering the prey item  
88 (Mathews et al., 2004); and 2) artificial prey items that act as lures and elicit a bite response  
89 by predators that can then be observed as marks on the lure surfaces. These are  
90 constructed out of materials such as modelling clay (Howe et al., 2009; Roslin et al., 2017;  
91 Mansion-Vaquié et al., 2017). Both approaches have the practical advantage of allowing  
92 studies to control prey densities and as such produce standardised assessments of  
93 predation that can be replicated a large number of times at a relatively low cost.

94

95 Several concerns have been raised about the different sentinel prey approaches. One of the  
96 most important is that immobilised or artificial prey no longer exhibit ecological mechanisms  
97 that play important roles in predation rates. For example, certain aphids show a dropping  
98 escape response to foliar-active natural enemies that can reduce predation rates (Losey &  
99 Denno, 1998a; Dixon, 1958). Additionally, the state (live, wounded, artificial or dead) of prey  
100 items has also been found to influence their attractiveness to predators (Zou et al., 2017;  
101 Ferrante et al., 2017). Such ecological mechanisms therefore have potential to impact on the  
102 level of predation recorded and consequently our capacity to infer pest control ecosystem  
103 services.

104

105 While different sentinel prey methods are currently widely used to infer predation rates, little  
106 is known about how many common predators respond to sentinel prey items and the manner  
107 in which they are presented. In this study we aim to address this issue by comparing the  
108 attack rates by two common predators: the Harlequin ladybird *Harmonia axyridis*  
109 (Coleoptera: Coccinellidae), and a carabid beetle *Pterostichus madidus* (Coleoptera:  
110 Carabidae) on immobilised prey aphids (*Sitobion avenae* (Hemiptera: Aphididae)) glued to  
111 card, and artificial prey (plasticine caterpillars). We compare attack rates on the sentinel  
112 methods and also assess how this differs to predation on live pest populations (free moving  
113 *S. avenae*) at two different predator densities in a mesocosm study system. We predicted:  
114 1) that an increase in attack rates on both sentinel prey (artificial caterpillars and immobilised  
115 aphids stuck to cards) and a reduction in live free moving aphid numbers (live pest  
116 population) in response to increasing predator density, under assumption that predator  
117 attack rates are a linear function of predator density (Abrams & Ginzburg 2000); 2) attack  
118 rates on immobilised aphids will be higher than on artificial prey, as the artificial prey do not  
119 possess any chemical cues used by both predator species to locate prey and do not  
120 represent a valid food item (Lövei & Sunderland, 1996; Kiehl et al., 1996; Abassi et al.,  
121 2000); and, 3) the ground beetle will be more likely than the ladybird to attack artificial prey  
122 as they have been shown to be highly opportunistic and generalist visual hunters (Lang &  
123 Gsödl, 2008; Ferrante et al., 2017). In contrast *H. axyridis* is highly dependent on olfactory  
124 as well as visual cues to locate prey (Koch, 2003).

125

## 126 **Methods**

### 127 **Experimental system**

128 We used an experimental mesocosm design to control predator density and composition  
129 between treatments. Each mesocosm comprised a 10L plant pot (28.5cm diameter / 22.5cm

130 deep), filled with peat-free compost and three wheat plants (*Triticum aestivum* L. Em. Fiori &  
131 Paol. Variety: KWS Dacanto), enclosed within a porous plastic mesh (height 36.5cm /  
132 diameter 28.5cm, pore size 0.05mm, held under standardised environmental conditions of  
133  $19.5 \pm 1^\circ\text{C}$  and LD 16:8 h). The combination of a ground-foraging predator, *P. madidus* and  
134 a foliar-foraging predator, *H. axyridis* was used as model predator community. Both species  
135 are predators of aphids, though have spatially segregated hunting niches (ground vs.  
136 canopy, respectively) (Schmitz, 2007; Woodcock & Heard, 2011). Adult *P. madidus* were  
137 collected through dry pitfall trapping and then stored in a controlled temperature facility ( $19.5$   
138  $\pm 1^\circ\text{C}$  and LD 16:8 h) in plastic cups containing moist soil, and were fed with dog food *ad*  
139 *libitum*. Within the same environment, adult *H. axyridis* were collected by hand from the field  
140 and stored in plastic 10L pots (28.5cm diameter / 22.5cm deep) covered with a porous  
141 plastic mesh (pore size 0.05mm) and were fed *ad libitum* with live aphids. Predators were  
142 kept for a maximum of four weeks in the laboratory. The pest species on which predation  
143 was assessed was *S. avenae*, an important aphid pest of wheat frequently used as a model  
144 prey item for measuring pest control (Mansion-Vaquié et al. 2017; Bøsem Baillod et al.  
145 2017). This aphid species shows a dropping behaviour in response to predator attacks  
146 (Winder 1990).

147

148 We tested two forms of sentinel prey commonly used to assess the delivery of natural pest  
149 control ecosystem services under field conditions. Immobilised prey represented by 10  
150 aphids glued using superglue (Loctite Super Glue, Henkel, Düsseldorf, Germany) to 4 x 6cm  
151 pieces of green card; aphids were placed approx., 0.5 cm apart. This reflects methodologies  
152 established by Winqvist et al. (2011). Within each mesocosm we suspended one card in the  
153 canopy of the wheat using a pin, and placed another on the soil surface of the plant pot  
154 (adapted from Winqvist et al. (2011)). We also used artificial prey designed to mimic  
155 lepidopteran caterpillars. Whilst the focus of our experiment was aphid prey, the use of  
156 artificial caterpillars has been widely used to infer predation rates in agricultural settings  
157 where the target pest species is not necessarily lepidopteran (Mansion-Vaquié et al. 2017).

158 Following approaches described in Howe, Lövei & Nachman (2009), caterpillars were made  
159 of non-toxic green plasticine (Newplast, Newclay, Devon, UK) and were 2cm × 0.5cm in  
160 diameter (Supplementary material: Appendix S1; Figure S1 & S2). Caterpillars were glued  
161 using superglue (Loctite) in pairs to 3x3cm pieces of green card. This ensured once  
162 constructed, no further handling of individual caterpillars occurred, avoiding the risk of  
163 accidental marks (important as marks were used as a measure of predation). A total of 10  
164 artificial prey items were suspended in the canopy by pinning the card with the caterpillars  
165 attached to the wheat foliage and 10 caterpillars placed on the soil surface, so the method  
166 could be quantitatively compared to the immobilised prey. In each mesocosm we also  
167 included live prey so that attack rates on the sentinel prey could be compared to live prey  
168 populations. Live prey populations were established as 20 free-moving adult *S. avenae*  
169 aphids evenly distributed on the leaves of each wheat plant. Aphids were allowed to settle  
170 for 4 hours, after which the two predator species were introduced. In addition to the two  
171 sentinel prey treatments, we also included a control treatment for each sentinel prey type  
172 that contained no predators. The control treatments were established following the same  
173 experimental protocol as above.

174

175 Using this model system, we assessed whether an increased density of predators resulted in  
176 higher attack rates on the sentinel prey and lower numbers of live aphids. We prepared a  
177 low-density treatment comprising two *H. axyrdis* and two *P. madidus*, and a high-predator  
178 density treatment with four individuals each of *H. axyrdis* and *P. madidus*. Each treatment  
179 was replicated seven times. All treatments were run at the same time with predators that  
180 were starved 24h prior to the experiment (predators were used only once i.e. a total of 84  
181 individuals of each species were used over the whole experiment). The proportion of  
182 immobilised aphids and the proportion of plasticine caterpillars showing evidence of attack  
183 were recorded out of 20 and the number of live aphids were counted after 24h from the point  
184 where predators were added.

185

## 186 **Statistical analysis**

187 We wanted to determine whether prey location (ground vs. foliar) affected predation rates at  
188 the two predator densities (low vs. high) and whether these attack rates differed between the  
189 sentinel prey methods (immobilised vs. artificial). However, we found no signs of predation  
190 by the ladybird on the artificial prey. This resulted in zero variation for this parameter which  
191 can lead to unreliable results in generalized linear models (Kuhn & Johnson 2013).  
192 Therefore, we first analysed the immobilised prey separately to determine whether attack  
193 rates differed between the ground and foliar predators at the different predator densities.  
194 Prey items were not analysed individually as statistically independent units, but rather a  
195 proportional attack rate across all 10 prey items at either the ground or foliage. We used a  
196 binomial distribution reflecting the bounding (0-1) of data. The response variable was attack  
197 rate (proportion of prey attacked out of 10) and the explanatory variables were predator  
198 density (low and high) and predator feeding location (ground or foliar) and the interaction  
199 between these two factors. As there were no predators in the controls for the artificial and  
200 immobilised prey treatments we found no signs of attack on the plasticine caterpillars or the  
201 aphids glued to cards (except one missing aphid from a card). This again meant that there  
202 was near zero variation for the controls and they were excluded from analysis. We then  
203 analysed the ground sentinel prey separately to determine whether *P. madidus* had higher  
204 attack rates on the plasticine caterpillars in comparison to the immobilised live prey, as it  
205 actively attacked both prey types. We used a binomial GLM with attack rate as the response  
206 variable and the explanatory variables predator density and prey type and the interaction  
207 between these two factors. Significance was assessed against a chi distribution.

208

209 To determine how predator density affected predation on live aphids we used a negative  
210 binomial GLM implemented in the MASS package (Venables & Ripley, 2002). A negative  
211 binomial GLM was used to account for overdispersion in the count data and for the fact that  
212 pest populations have the capacity to reproduce, even over a 24h time frame. The response



213 variable was the number of aphids counted in the mesocosm at the end of the experiment  
214 and the explanatory variables were predator density (control (no predators), low and high)  
215 and alternative prey type (artificial and immobilised) and the interaction between these two  
216 factors. Significance was assessed against a chi distribution. Where the interaction was  
217 significant, orthogonal post-hoc contrasts were carried out. All analyses were carried out in R  
218 (R Core Team, 2017).

219

## 220 Results

221 For both sentinel prey methods, evidence of attack was recorded after the 24h foraging  
222 period, suggesting that immobilised prey stuck to cards and artificial caterpillars elicited a  
223 predation response in the predators. However, we found no signs of predation on the  
224 artificial caterpillars in the canopy and found no jaw marks from the predator *H. axyridis* on  
225 any of the artificial caterpillars placed on the soil surface; these showed predation only by *P.*  
226 *madidus*. Of the immobilised aphid prey, we found no significant interaction between  
227 predator feeding location and predator density on predator attack rates ( $\chi^2 = 0.210$ ,  $df = 1$ ,  $p$   
228  $= 0.647$ ). Predator feeding location was also not found to have a significant effect on attack  
229 rates on the immobilised prey ( $\chi^2 = 1.981$ ,  $df = 1$ ,  $p = 0.159$ ), however there was a significant  
230 effect of predator density ( $\chi^2 = 10.407$ ,  $df = 1$ ,  $p = 0.002$ ). Attack rates were significantly  
231 higher at the high predator density compared to the low predator density (proportion of prey  
232 attacked out of 10 on immobilised prey: low predator density =  $0.207 \pm 1SE 0.046$ ; high  
233 predator density =  $0.779 \pm 1SE 0.094$ ). Where predation was compared between sentinel  
234 prey types for *P. madidus* we found there was no significant interaction between prey type  
235 and predator density ( $\chi^2 = 0.269$ ,  $df = 1$ ,  $p = 0.604$ ). Prey type was also not significant ( $\chi^2 =$   
236  $0.020$ ,  $df = 1$ ,  $p = 0.887$ ), however there was a significant increase in attack rates by *P.*  
237 *madidus* between predator densities ( $\chi^2 = 10.080$ ,  $df = 1$ ,  $p = 0.001$ ) (low predator density  
238 mean =  $0.114 \pm 1SE 0.038$ ; high predator density mean =  $0.679 \pm 1SE 0.070$ ).

239

240 There was no significant interaction between predator density and alternative prey type on  
241 the number of live prey in each treatment ( $\chi^2 = 1.110$ ,  $df = 1$ ,  $p = 0.574$ ), however both main  
242 effects predator density and alternative prey type were significant (Alternative prey type  $\chi^2 =$   
243  $6.066$ ,  $df = 1$ ,  $p = 0.014$ ; Predator density  $\chi^2 = 21.813$ ,  $df = 2$ ,  $p = <0.001$ ). Post hoc  
244 comparisons showed that there was a significant difference between the number of live  
245 aphids in the control treatments and the predation treatments ( $z = -4.521$ ,  $p = <0.001$ ) (Table  
246 1). However, there was no significant difference between the low predator density treatment  
247 and high predator density treatment ( $z = 1.100$ ,  $p = 0.271$ ). The number of live aphids in the  
248 artificial prey treatment was significantly lower than the immobilised prey treatment (Table 1).

## 249 Discussion

### 250 **Effect of predator density on attack rates**

251 In accordance with our first prediction, both the immobilised and artificial prey detected  
252 increased attack rates in response to a higher predator density. However, in the case of the  
253 live aphids there was no evidence of increased consumption at the higher predator densities.  
254 This contrasts with the higher attack rates seen for the sentinel prey under the same  
255 conditions. The sentinel prey represented both aggregated and highly conspicuous prey that,  
256 in contrast to the live aphids, were unable to escape from predators. In this situation, once  
257 the predators located the prey the two predominant limiting effects on attack rates would be  
258 predator satiation or negative intraguild interactions (Gagnon, Heimpel & Brodeur 2011).  
259 Immobilised prey were viable food items, so would contribute to predator satiation, which  
260 could have reduced predation on the free moving prey (the number of free moving aphids  
261 was still significantly lower in the predation treatments compared to the control, indicating  
262 that predation did occur on the live pest populations). In contrast, the artificial prey is unlikely  
263 to contribute to predator satiation as it offers no nutrition, which could lead to an inflation of  
264 attack rates on artificial prey (where predators continually attack the prey due to a lack of  
265 satiation) or cause them to search for alternative prey (Lövei & Ferrante 2017). We found  
266 that significantly more free-moving aphids were consumed in the artificial prey treatment

267 compared to the immobilised prey treatment, suggesting the predators were attacking the  
268 live prey to gain food (although the number of aphids consumed did not change as a  
269 function of predator density). However, there was strong evidence that at the higher predator  
270 densities artificial caterpillars were often attacked multiple times (i.e >40% of caterpillars  
271 were attacked). This relatively high attack rate for the artificial caterpillars on the ground may  
272 have reduced predation by the ground foraging beetles on the live aphids. A final point is  
273 that in comparison to the sentinel prey, the free-moving aphids would be able to avoid  
274 predators through either escape responses such as dropping from the plant when attacked,  
275 or persisting in refuges where they are less vulnerable to predation; both mechanisms have  
276 been found to reduce predation rates (Losey & Denno, 1998a; b; Berryman et al., 2006;  
277 Bommarco et al., 2007). This could also explain the lower levels of predation on the free-  
278 moving aphid populations also included in the mesocosm.

279

## 280 **Predation responses to the different sentinel prey methods**

281 We found equivocal evidence in support of our second prediction that attack rates were  
282 lower on the artificial prey compared to the immobilised prey, with no significant difference in  
283 attack rates by *P. madidus* being identified between the sentinel prey. However, if predation  
284 assessments were based only on the artificial caterpillars located in the plant canopy, then  
285 no difference in predation would have been detected as *H. axyridis* was not seen to bite  
286 these artificial prey items. This agrees with the findings of Lövei and Ferrante, (2017) who  
287 demonstrated lower predation on artificial sentinel prey compared to real sentinel prey. Our  
288 results suggest this is due to individual predator feeding preferences. The lack of predation  
289 by *H. axyridis* supported our third prediction that ground beetles would be more likely to  
290 attack artificial prey. Both ground beetles and ladybirds have been found to use visual cues  
291 when selecting feeding patches (e.g. attracted to high prey densities) (Lövei & Sunderland,  
292 1996; Osawa, 2000; Lee & Kang, 2004) and both groups have also been found to respond  
293 to and locate prey based on aphid volatiles (Lövei & Sunderland, 1996; Kielty et al., 1996;

294 Koch, 2003). However, our results suggest that either *H. axyridis* does not view plasticine  
295 caterpillars as a prey item, or demonstrates preferences for live aphids over lepidopteran  
296 prey. It is worth noting here that *H. axyridis* is polyphagous and will feed on juvenile stages  
297 of Lepidoptera (Koch et al., 2003). For this reason other factors may also contribute to the  
298 effective avoidance of the artificial caterpillars by *H. axyridis*. For example, *H. axyridis* relies  
299 more on olfactory cues and has been shown to be highly attracted to the chemical (*E*)- $\beta$ -  
300 farnesene a key component of the alarm pheromone for most aphid species including *S.*  
301 *avenae* (Verheggen et al., 2007). In contrast, ground beetles are more opportunistic  
302 predators and may base feeding choices on prey vulnerability (Lang & Gsödl, 2008), which  
303 could increase the likelihood of ground beetles attacking artificial prey items. The use of  
304 plasticine caterpillars may therefore be a poor measure of predation where the dominant  
305 predators in the ecosystem are Coccinellidae or other taxa that show similar hunting  
306 strategies.

307

308 Sentinel methods are rarely used to calculate absolute predation and are more frequently  
309 used to compare the relative amount of predation between experimental units (Lövei &  
310 Ferrante, 2017). When combined with information on crop yield, direct measures of pest  
311 damage and conventional quantification of both pest and predator densities, sentinel prey  
312 approaches have the potential to provide valuable insights into pest control dynamics in  
313 agro-ecosystems. Whilst understanding relative changes in predation between experimental  
314 units is useful in elucidating ecosystems dynamics, being able to use sentinel prey items to  
315 provide a surrogate measure of pest control for target pest species could be developed into  
316 a standardised measure of pest control that can be applied to a range of farming systems.  
317 Our study provides a basic demonstration that live and sentinel prey items detect varying  
318 levels of predation in response to different predator species and predator densities, which  
319 highlights potential limitations of using sentinel prey as proxies for pest suppression.  
320 However, as live prey populations are able to reproduce and move, dynamics which cannot

321 be replicated in sentinel prey, the measure of success for real prey is often based on pest  
322 threshold densities. As such it is very difficult to draw parallels between predation on live  
323 and sentinel prey items.

324

325 There are limitations in this study to the sentinel prey approaches used to evaluate natural  
326 pest control that merit consideration if the findings of this research are to inform future work.  
327 Firstly, the number of artificial caterpillars may have been unrealistically high as this prey  
328 item was included in mesocosms at the same density as the immobilised aphids. This was  
329 done in part for practical reasons; if the number of prey were too small then detectable  
330 differences between experimental units would be hard to observe, particular where all the  
331 prey were consistently attacked or consumed (a problem akin to the “ceiling effect” in  
332 statistics) (Austin & Brunner 2003). However, comparable densities to the immobilised prey  
333 used in this study are not uncommon for pest populations under field conditions. For  
334 example, caterpillars such as *Artogeia rapae* (small cabbage white) can reach similar  
335 densities (Hooks, Pandey & Johnson 2003), while aggregations of aphids will normally  
336 exceed those used in this study (Sunderland & Vickerman 1980; Sopp, Sunderland &  
337 Coombes 1987). Secondly, the sentinel prey types in our study could have been assessed in  
338 isolation without alternative real prey. This would have enabled the relative differences in  
339 predation between methods to be directly compared more easily. However, to understand  
340 how these methods perform in the real-world, where predators are exposed to both naturally  
341 occurring free-moving and experimental sentinel prey, using more than one prey species  
342 provides a more realistic comparison. In mesocosms a predator may attack the sentinel prey  
343 (where it is the only prey) out of necessity (starvation), which directly contrasts to an  
344 agroecosystem where alternative prey are likely to be available. Accordingly, this could  
345 falsely represent predation by that species on sentinel prey. A similar criticism could also be  
346 made where studies use a single real prey species to assess natural pest control. However,  
347 typically these studies focus on a model prey species deliberately selected as it represents a

348 pest of economic importance to that crop. In this situation avoidance of that key pest species  
349 in preference to alternative prey still provides key biologically relevant information in terms of  
350 assessing pest control, at least for that key pest. Finally, it is possible that due to the close  
351 spacing of the prey, that that the free-moving aphids could walk on the caterpillars and  
352 potentially leave prey-related chemical cues on them increasing their level of attraction to the  
353 predators. However, we found that more aphids were consumed where the alternate prey  
354 were the plasticine caterpillars as opposed to the immobilised prey. This would suggest that  
355 the predators were distinguishing between the artificial prey and real prey in the mesocosm  
356 without being affected by such chemical cues.

357

## 358 Conclusion

359 Sentinel prey methods offer a simple way to measure predation that have significant  
360 advances over surrogate measures that rely on variation in prey or predator abundances  
361 (Lövei & Sunderland, 1996; Chaplin-Kramer et al., 2011; Lövei & Ferrante, 2017). However,  
362 when using sentinel prey our results highlight the importance of considering the effects of  
363 predator and pest species life-history traits and the influence these have on observed  
364 predation. A sensible approach would be to consider more than one measure of prey  
365 suppression and tailor it to the desired measure of the study (Macfadyen et al., 2015). For  
366 example, using plasticine caterpillars in conjunction with live, free moving prey (of a known  
367 focal pest species) would allow a practitioner to record potential key predators within an  
368 ecosystem based on the detection of bite marks in the plasticine, whilst also giving an  
369 indication of actual pest suppression on the live prey. Correlation in predation rates between  
370 the two methods could be used to determine whether the predators revealed by the artificial  
371 prey method are the dominant predators responsible for pest control within that particular  
372 agroecosystem. Accounting for variation in the attractiveness of different prey items to  
373 predators, the effects of inhibiting important ecological escape mechanisms and the effects

374 of different sentinel prey densities will improve estimates of prey suppression. Ultimately,  
375 this will improve our understanding of how natural pest control is delivered under field  
376 conditions.

377

## 378 **Author contribution**

- 379 • All authors conceived the research
- 380 • Author 1 and author 2 designed and conducted experiments
- 381 • Author 1 conducted statistical analyses
- 382 • Author 1 wrote the draft manuscript
- 383 • All authors contributed to writing the final and revised manuscripts
- 384 • All authors approved the final version of the manuscript

385

386

## 387 **References**

- 388 Abassi SA, Birkett MA, Pettersson J, Pickett JA, Wadhams LJ & Woodcock CM. (2000).  
389 Response of the Seven-spot Ladybird to an Aphid Alarm Pheromone and an Alarm  
390 Pheromone Inhibitor is Mediated by Paired Olfactory Cells. *Journal of Chemical Ecology*,  
391 26:1765-1771
- 392 Abrams, P.A. & Ginzburg, L.R. (2000) The nature of predation: prey dependent, ratio  
393 dependent or neither? *Trends in Ecology & Evolution*, 15, 337–341.
- 394 Austin, P.C. & Brunner, L.J. (2003) Type I error inflation in the presence of a ceiling effect.  
395 *The American Statistician*, 57, 97–104.
- 396 Begg, G.S., Cook, S.M., Dye, R., Ferrante, M., Franck, P., Lavigne, C., Lövei, G. Mansion-  
397 Vaquie, A., Pell, J., K., Petit, S., Quesada, N., Ricci, B. & Birch, A.N.E (2017) A functional  
398 overview of conservation biological control. *Crop Protection* 97: 145-158
- 399 Berryman AA, Hawkins BA & Hawkins BA. (2006). The refuge as an integrating concept in  
400 ecology and evolution. *Oikos*, 115:192–196.
- 401 Bianchi FJJA, Booij CJH & Tschardt T. (2006). Sustainable pest regulation in agricultural  
402 landscapes: a review on landscape composition, biodiversity and natural pest control.  
403 *Proceedings. Biological Sciences / the Royal Society*, 273:1715–1727.
- 404 Bianchi FJJA, van Wingerden WKRE, Griffioen AJ, van der Veen M, van der Straten MJJ,  
405 Wegman RMA & Meeuwsen HAM. (2005). Landscape factors affecting the control of

406 *Mamestra brassicae* by natural enemies in Brussels sprout. *Agriculture, Ecosystems &*  
407 *Environment*, 107:145–150.

408 Birkhofer K, Bylund H, Dalin P, Ferlian O, Gagic V, Hambäck PA, Klapwijk M, Mestre L,  
409 Roubinet E, Schroeder M, Stenberg JA, Porcel M, Björkman C & Jonsson M. (2017).  
410 Methods to identify the prey of invertebrate predators in terrestrial field studies. *Ecology and*  
411 *Evolution*, 7:1942–1953.

412 Bommarco R, Firlé SO & Ekbom B. (2007). Outbreak suppression by predators depends on  
413 spatial distribution of prey. *Ecological Modelling*, 201:163–170.

414 Bosem Baillod, A., Tscharrntke, T., Clough, Y. and Batáry, P. (2017). Landscape-scale  
415 interactions of spatial and temporal cropland heterogeneity drive biological control of cereal  
416 aphids. *Journal of Applied Ecology*, 54:1804-1813.

417 Cardinale BJ, Srivastava DS, Duffy JE, Wright JP, Downing AL, Sankaran M & Jouseau C.  
418 (2006). Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature*,  
419 443:989–992.

420 Chaplin-Kramer R, O'Rourke ME, Blitzer EJ & Kremen C. (2011). A meta-analysis of crop  
421 pest and natural enemy response to landscape complexity. *Ecology Letters*, 14:922–932.

422 Dixon AFG. (1958). The escape responses shown by certain aphids to the presence of the  
423 coccinellid *Adalia decempunctata* (L.). *Transactions of the Royal Entomological Society of*  
424 *London*, 110:319–334.

425 Elliott NC, Kieckhefer RW, Lee J-H & French BW. (1999). Influence of within-field and  
426 landscape factors on aphid predator populations in wheat. *Landscape Ecology* 14:239-252.

427 Ferguson KI & Stiling P. (1996). Non-additive effects of multiple natural enemies on aphid  
428 populations. *Oecologia*, 108:375–379.

429 Ferrante M, Barone G & Lövei GL. (2017). The carabid *Pterostichus melanarius* uses  
430 chemical cues for opportunistic predation and saprophagy but not for finding healthy prey.  
431 *Biocontrol*, 62:741–747.

432 Foster WA, Snaddon JL, Turner EC, Fayle TM, Cockerill TD, Ellwood MDF, Broad GR,  
433 Chung AYC, Eggleton P, Khen CV & Yusah KM. (2011). Establishing the evidence base for  
434 maintaining biodiversity and ecosystem function in the oil palm landscapes of South East  
435 Asia. *Philosophical Transactions of the Royal Society of London. Series B, Biological*  
436 *Sciences*, 366:3277–3291.

437 Gagnon, A.-È., Heimpel, G.E. & Brodeur, J. (2011) The ubiquity of intraguild predation  
438 among predatory arthropods. *Plos One*, 6, e28061.

439 Gardiner MM, Landis DA, Gratton C, DiFonzo CD, O'Neal M, Chacon JM, Wayo MT,  
440 Schmidt NP, Mueller EE & Heimpel GE. (2009). Landscape diversity enhances biological  
441 control of an introduced crop pest in the north-central USA. *Ecological Applications*, 19:143–  
442 154.

443 Greenop A, Woodcock BA, Wilby A, Cook SM & Pywell RF. (2018). Functional diversity  
444 positively affects prey suppression by invertebrate predators: a meta-analysis. *Ecology*,  
445 doi.org/10.1002/ecy.2378



446 Holland JM, Oaten H, Moreby S, Birkett T, Simper J, Southway S & Smith BM. (2012). Agri-  
447 environment scheme enhancing ecosystem services: A demonstration of improved biological  
448 control in cereal crops. *Agriculture, Ecosystems & Environment*, 155:147–152.

449 Hooks, C.R.R., Pandey, R.R. & Johnson, M.W. (2003) Impact of avian and arthropod  
450 predation on lepidopteran caterpillar densities and plant productivity in an ephemeral  
451 agroecosystem. *Ecological Entomology*, 28, 522–532.

452 Howe A, Lövei GL & Nachman G. (2009). Dummy caterpillars as a simple method to assess  
453 predation rates on invertebrates in a tropical agroecosystem. *Entomologia experimentalis et*  
454 *applicata*, 131:325–329.

455 Kiely JP, Allen-Williams LJ, Underwood N & Eastwood EA. (1996). Behavioral responses of  
456 three species of ground beetle (Coleoptera: Carabidae) to olfactory cues associated with  
457 prey and habitat. *Journal of Insect Behavior*, 9:237–250.

458 Koch RL, Hutchison WD, Venette RC & Heimpel GE. (2003). Susceptibility of immature  
459 monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae: Danainae), to predation by  
460 *Harmonia axyridis* (Coleoptera: Coccinellidae). *Biological Control*, 28:265–270.

461 Koch RL. (2003). The multicolored Asian lady beetle, *Harmonia axyridis*: A review of its  
462 biology, uses in biological control, and non-target impacts. *Journal of Insect Science*, 3:1–16.

463 Kuhn, M. & Johnson, K. (2013) *Applied Predictive Modeling*. Springer New York, New York,  
464 NY.

465 Lang A & Gsödl S. (2008). Prey vulnerability and active predator choice as determinants of  
466 prey selection: a carabid beetle and its aphid prey. *Journal of Applied Entomology*, 125:53–  
467 61.

468 Lee J-H & Kang T-J. (2004). Functional response of *Harmonia axyridis* (Pallas) (Coleoptera:  
469 Coccinellidae) to *Aphis gossypii* Glover (Homoptera: Aphididae) in the Laboratory. *Biological*  
470 *Control*, 31:306–310.

471 Losey JE & Denno RF. (1998a). The escape response of pea aphids to foliar-foraging  
472 predators: factors affecting dropping behaviour. *Ecological Entomology* 23:53–61.

473 Losey JE & Denno RF. (1998b). Interspecific variation in the escape responses of aphids:  
474 effect on risk of predation from foliar-foraging and ground-foraging predators. *Oecologia*,  
475 115:245–252.

476 Lövei GL & Ferrante M. (2017). A review of the sentinel prey method as a way of quantifying  
477 invertebrate predation under field conditions. *Insect Science*, 24:528–542.

478 Lövei GL & Sunderland KD. (1996). Ecology and behavior of ground beetles (Coleoptera:  
479 Carabidae). *Annual Review of Entomology*, 41:231–256.

480 Macfadyen S, Davies AP & Zalucki MP. (2015). Assessing the impact of arthropod natural  
481 enemies on crop pests at the field scale. *Insect Science*, 22:20–34.

482 Mansion-Vaquié A, Ferrante M, Cook SM, Pell JK & Lövei GL. (2017). Manipulating field  
483 margins to increase predation intensity in fields of winter wheat (*Triticum aestivum*). *Journal*  
484 *of Applied Entomology*, 141:600–611.

485 Mathews CR, Bottrell DG & Brown MW. (2004). Habitat manipulation of the apple orchard  
486 floor to increase ground-dwelling predators and predation of *Cydia pomonella* (L.)  
487 (Lepidoptera: Tortricidae). *Biological Control*, 30:265–273.

488 Northfield TD, Snyder GB, Ives AR & Snyder WE. (2010). Niche saturation reveals resource  
489 partitioning among consumers. *Ecology Letters*, 13:338–348.

490 Osawa N. (2000). Population field studies on the aphidophagous ladybird beetle *Harmonia*  
491 *axyridis* (Coleoptera: Coccinellidae): resource tracking and population characteristics.  
492 *Population Ecology*, 42:115–127.

493 R Core Team (2017). R: A language and environment for statistical computing. R  
494 Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

495 Roslin T, Hardwick B, Novotny V, Petry WK, Andrew NR, Asmus A, Barrio IC, Basset Y,  
496 Boesing AL, Bonebrake TC, Cameron EK, Dáttilo W, Donoso DA, Drozd P, Gray CL, Hik  
497 DS, Hill SJ, Hopkins T, Huang S, Koane B, Laird-Hopkins B, Laukkanen L, Lewis OT, Milne  
498 S, Mwesige I, Nakamura A, Nell CS, Nichols E, Prokurat A, Sam K, Schmidt NM, Slade A,  
499 Slade V, Suchanková A, Teder T, van Nouhuys S, Vandvik V, Weissflog A, Zhukovich V &  
500 Slade EM. (2017). Higher predation risk for insect prey at low latitudes and elevations.  
501 *Science*. 356:742–744.

502 Rusch A, Chaplin-Kramer R, Gardiner MM, Hawro V, Holland J, Landis D, Thies C,  
503 Tschardtke T, Weisser WW, Winqvist C, Woltz M & Bommarco R. (2016). Agricultural  
504 landscape simplification reduces natural pest control: A quantitative synthesis. *Agriculture,*  
505 *Ecosystems & Environment*, 221:198–204.

506 Schmidt MH, Roschewitz I, Thies C & Tschardtke T. (2005). Differential effects of landscape  
507 and management on diversity and density of ground-dwelling farmland spiders. *Journal of*  
508 *Applied Ecology*, 42:281–287.

509 Schmitz OJ. (2007). Predator diversity and trophic interactions. *Ecology*, 88:2415–2426.

510 Snyder GB, Finke DL & Snyder WE. (2008). Predator biodiversity strengthens aphid  
511 suppression across single- and multiple-species prey communities. *Biological Control*,  
512 44:52–60.

513 Sopp, P.I., Sunderland, K.D. & Coombes, D.S. (1987) Observations on the number of cereal  
514 aphids on the soil in relation to aphid density in winter wheat. *Annals of Applied Biology*, 111,  
515 53–57.

516 Straub CS & Snyder WE. (2006). Species identity dominates the relationship between  
517 predator biodiversity and herbivore suppression. *Ecology*, 87:277–282.

518 Sunderland, K.D. & Vickerman, G.P. (1980) Aphid feeding by some polyphagous predators  
519 in relation to aphid density in cereal fields. *The Journal of Applied Ecology*, 17, 389.

520 Venables WN & Ripley BD. (2002). Modern Applied Statistics with S. Statistics and  
521 Computing. (ed by J Chambers, W Eddy, W Härdle, S Sheather & L Tierney) 4th edn.  
522 Springer New York, New York, NY,.

523 Verheggen FJ, Fagel Q, Heuskin S, Lognay G, Francis F & Haubruge E. (2007).  
524 Electrophysiological and behavioral responses of the multicolored Asian lady beetle,  
525 *Harmonia axyridis pallas*, to sesquiterpene semiochemicals. *Journal of Chemical Ecology*,  
526 33:2148–2155.

527 Wan, N.-F., Ji, X.-Y., Kiær, L.P., Liu, S.-S., Deng, J.-Y., Jiang, J.-X. & Li, B. (2018) Ground  
528 cover increases spatial aggregation and association of insect herbivores and their predators  
529 in an agricultural landscape. *Landscape Ecology*, 33, 799–809.

530 Winder, L. (1990). Predation of the cereal aphid *Sitobion avenae* by polyphagous predators  
531 on the ground. *Ecological Entomology*, 15:105-110.

532 Winder, L., Perry, J.N. & Holland, J.M. (1999) The spatial and temporal distribution of the  
533 grain aphid *Sitobion avenae* in winter wheat. *Entomologia experimentalis et applicata*, 93,  
534 275–288.

535 Winqvist C, Bengtsson J, Aavik T, Berendse F, Clement LW, Eggers S, Fischer C, Flohre A,  
536 Geiger F, Liira J, Pärt T, Thies C, Tschardt T, Weisser WW & Bommarco R. (2011). Mixed  
537 effects of organic farming and landscape complexity on farmland biodiversity and biological  
538 control potential across Europe. *Journal of Applied Ecology*, 48:570–579.

539 Woodcock B., Bullock J., McCracken M, Chapman R., Ball S., Edwards M., Nowakowski M  
540 & Pywell R. (2016). Spill-over of pest control and pollination services into arable crops.  
541 *Agriculture, Ecosystems & Environment*, 231:15–23.

542 Woodcock BA & Heard MS. (2011). Disentangling the effects of predator hunting mode and  
543 habitat domain on the top-down control of insect herbivores. *The Journal of Animal Ecology*,  
544 80:495–503.

545 Zalucki MP, Furlong MJ, Schellhorn NA, Macfadyen S & Davies AP. (2015). Assessing the  
546 impact of natural enemies in agroecosystems: toward “real” IPM or in quest of the Holy  
547 Grail? *Insect Science*, 22:1–5.

548 Zou Y, de Kraker J, Bianchi FJJA, van Telgen MD, Xiao H & van der Werf W. (2017). Video  
549 monitoring of brown planthopper predation in rice shows flaws of sentinel methods. *Scientific*  
550 *Reports*, 7:42210.

551

552

553

554

555

556

557

558

559

560

561

562

563

564

565

566

567 **Table 1.** Number of live aphids (*Sitobion avenae*) recorded after 24h exposure to the  
 568 predators *Pterostichus madidus* and *Harmonia axyridis* in a mesocosm where either  
 569 immobilised prey (20 *S. avenae* aphids glued to card) or artificial prey (20 plasticine  
 570 caterpillars) were also available. Predator densities control (no predators), low (two *H.*  
 571 *axyridis* and two *P. madidus*) and high (four *H. axyridis* and four *P. madidus*) are the mean  
 572 across both alternate prey types. Artificial prey treatment and immobilised prey treatment are  
 573 the mean across all predator densities.

<b>Treatment</b>	<b>Number of live aphids (mean <math>\pm</math>1SE)</b>
Control	28.786 $\pm$ 2.823
Low predatory density	18.429 $\pm$ 2.336
High predator density	15.643 $\pm$ 1.561
Artificial prey treatment	18.190 $\pm$ 2.102
Immobilised prey treatment	23.714 $\pm$ 2.212

574

575