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Escape from parasitism by the invasive alien ladybird, *Harmonia axyridis*

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Abstract:

1. Alien species are often reported to perform better than functionally-similar species native to the invaded range, resulting in high population densities, and a tendency to become invasive. The Enemy Release Hypothesis (ERH), explains the success of invasive alien species (IAS) as a consequence of reduced mortality from natural enemies (predators, parasites and pathogens) compared to native species. The harlequin ladybird, *Harmonia axyridis,* a species alien to Britain, provides a model system for testing the ERH.

2. Pupae of *H. axyridis* and the native ladybird *Coccinella septempunctata* were monitored for parasitism between 2008 and 2011, from populations across southern England in areas first invaded by *H. axyridis* between 2004 and 2009. Additionally, a semi-field experiment was established to investigate the incidence of parasitism of adult *H. axyridis* and *C. septempunctata* by *Dinocampus coccinellae*.

3. *Harmonia axyridis* pupae were parasitised at a much lower rate than conspecifics in the native range, and both pupae and adults were parasitised at a considerably lower rate than *C. septempunctata* populations from the same place and time (*H. axyridis*: 1.67%; *C. septempunctata*: 18.02%) or in previous studies on Asian *H. axyridis* (2-67%). We found no evidence that the presence of *H. axyridis* affected the parasitism rate of *C. septempunctata* by *D. coccinellae*.

4. Our results are consistent with the general prediction that the prevalence of natural enemies is lower for introduced species than for native species at early stages of invasion. This may partly explain why *H. axyridis* is such a successful IAS.

Keywords: enemy release hypothesis, natural enemies, native species, invasive alien species, *Harmonia axyridis*, *Coccinella septempunctata*

Introduction:

Biological invasions are a major threat to native ecosystems (Millennium Ecosystem Assessment, 2005, UK National Ecosystem Assessment, 2011), and the rate of establishment of alien species is accelerating (Roy, Bacon, Beckmann *et al.*, 2012b, Roy, Roy & Roques, 2011). Not all the species introduced to an area establish: 10% is commonly acknowledged as the proportion of new arrivals that establish successfully, but this is based on an assessment of plant species in Britain (Williamson & Fitter, 1996) and there is evidence that this rate may not hold true for other taxa (Jeschke, Aparicio, Haider *et al.*, 2012, Jeschke, 2008).

Some introduced species have become spectacularly successful in the new regions they occupy, and are termed invasive alien species (IAS) in view of their rapid spread and high impact on native diversity. Many new arrivals do less well, failing to establish or surviving only as small, isolated populations (Lodge, 1993). One commonly-cited potential explanation for this discrepancy is the Enemy Release Hypothesis (ERH) (Elton, 1958, Torchin, Lafferty, Dobson *et al.*, 2003), also known as enemy-escape (Brown, Abrahamson, Packer *et al.*, 1995) or the escape-from-enemy hypothesis (Wolfe, 2002). This predicts that an alien species introduced to a new region will experience reduced mortality from specialised natural enemies (e.g., predators, parasites and pathogens) compared to native species. The co-evolved natural enemy species remain in the native range of the IAS, while natural enemies native to the introduced range of the IAS will not be co-evolved with it, and will often take time to adapt to a novel prey or host species. This gives the IAS a competitive advantage, assuming that natural enemies are important in regulating populations (Roy & Lawson Handley, 2012c), and allows a rapid increase in the abundance and distribution of the alien species (Elton, 1958, Torchin *et al.*, 2003, Colautti, Ricciardi, Grigorovich *et al.*, 2004).

Two mechanisms have been proposed to account for the increase in population growth of the alien species in comparison to the native species: regulatory or compensatory release (Colautti et al. 2004). For hosts that are strongly regulated by enemies in their native range a reduction in enemies in the introduced range may lead to direct changes in survivorship, fecundity, biomass or other parameters (regulatory release). Alternatively, for hosts that are well-defended and, consequently, lack natural enemies within their native range, a reduction in enemies may be of minimal consequence for hosts. Indeed for well-defended hosts, fewer enemies may lead to a reallocation of resources from defence to population growth over ecological time (Roy *et al.*, 2012c), so-called compensatory release or as the Evolution of Increased Competitive Ability (EICA). Empirical evidence for the role of the ERH or EICA in invasion success is lacking (Roy et al. 2011), particularly for invertebrates,

The harlequin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), is considered to be a highly successful IAS (Roy, Brown & Majerus, 2006, Brown, Adriaens, Bathon *et al.*, 2008a, Roy & Wajnberg, 2008b). It is native to temperate Asia, but is now established across Europe, Africa, and both North and South America (Brown, Thomas, Lombaert *et al.*, 2011b). There has often been a time lag between introduction of *H. axyridis*, as a biological control agent for aphids and coccids, and establishment (Koch, 2003). This species is also known to colonise through natural spread but also anthropogenically on produce and along transport networks (Brown, Adriaens, Bathon *et al.*, 2008b). *Harmonia axyridis* established in Britain in 2004 (Majerus, Mabbott, Rowland *et al.*, 2006), and spread at more than 100 km per year (Brown, Roy, Rothery *et al.*, 2008), contributing to declines in several native species (Roy, Adriaens, Isaac *et al.*, 2012a, Ware & Majerus, 2008, Brown, Frost, Doberski *et al.*, 2011a) most likely through competition and predation (Ware & Majerus, 2008).

The invasive nature of *H. axyridis* is thought to result from several factors. The species has good dispersal capabilities, occupies a broad range of habitats, has high reproductive potential, broad climatic tolerance, and a wide dietary range, including a propensity to act as an intraguild predator within the aphidophagous guild (Majerus *et al.*, 2006, Ware, Majerus, Roy *et al.*, 2005, Roy & Wajnberg, 2008a, Berkvens, Baverstock, De Clercq *et al.*, 2008, Brown *et al.*, 2011b, Soares, Borges, Borges *et al.*, 2008). As a well-defended species, with strong chemical defences and large larval spines (Sloggett, Magro, Verheggen *et al.*, 2011, Ware *et al.*, 2008) introduced to a new continent many thousands of kilometres from its native range, there is also a strong possibility that enemy release plays a role in the success of *H. axyridis*.

In Britain, several species of endoparasitic Hymenoptera and Diptera attack ladybirds. Some of these have a Holarctic distribution, and are known to attack *H. axyridis* in its native range: *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) (Kuznetsov, 1997, Ware, Michie, Otani *et al.*, 2010); *Homalotylus flaminius* Dalman (Hymenoptera: Encyrtidae) (Kuznetsov, 1997); *Oomyzus scaposus* (=*Tetrastichus coccinellae*) Thomson (Hymenoptera: Eulophidae) (Kuznetsov, 1997) and *Medina separata* (Meigen) (Diptera: Tachinidae) (Kuznetsov, 1997) (often erroneously referred to as *M. luctuosa* (Hodek, van Emden & Honek, 2012)), while others (*Phalacrotophora fasciata* (Fallén) and *Phalacrotophora berolinensis* Schmitz (Diptera: Phoridae) (Disney & Beuk, 1997, Disney et al., 1994)) are European in distribution but are closely related to *Phalacrotophora philaxyridis* Disney (Diptera: Phoridae) which attacks *H. axyridis* in Japan (Disney, 1997).

There is evidence that some natural enemies of ladybirds, particularly the holarctic species known to attack *H. axyridis* in Asia, are beginning to attack it in Britain too (Ware *et al.*, 2010, Hall, Ware & Michie, 2009). It is unclear what effect this will have on native ladybird species. As parasitoids are shared across ladybird species, there is the potential for apparent competition (Holt, 1977, Bonsall & Hassell, 1997), where high abundance of *H. axyridis* elevates rates of parasitism in susceptible native species. Alternatively, *H. axyridis* may act as a parasitoid sink, whereby the parasitoid may oviposit in the IAS but the eggs do not produce an adult, potentially reducing the population density of the parasitoid and thus the parasite burden on native populations. This has been suggested for the parasitoid wasp *D. coccinellae*, which in laboratory studies oviposited approximately equally into *H. axyridis* and *Coccinella septempunctata* L., the primary native host in Britain, but which successfully eclosed from a significantly greater proportion of *C. septempunctata* (Koyama & Majerus, 2008). Additionally a study comparing parasitism of *Coleomegilla maculata* De Geer with that of *H. axyridis* by *D. coccinellae* also concluded that *H. axyridis* was an unsuitable host (Hoogendoorn & Heimpel, 2002). Teratocyte cells, produced by *D. coccinellae*, are involved in immunosuppression of the host and nutrition of the parasitoid, interestingly follow an abnormal pattern of growth within *H. axyridis* which could explain the impeded development of *D. coccinellae* within this marginal host (Firlej, 2012). Therefore, it is unlikely that *D. coccinellae* will limit the population growth of *H. axyridis* within invaded ranges (Berkvens, Moensa, Berkvens *et al.*, 2010, Hoogendoorn *et al.*, 2002). Intriguingly a recent study demonstrated that *H. axyridis* individuals contain high numbers of obligate parasitic microsporidia which while not seemingly harmful to *H. axyridis* are lethal when artificially injected into the native ladybird *C. septempunctata* (Vilcinskas, Stoecker, Schmidtberg *et al.*, 2013).

In this paper, we examine the following hypotheses:

- Time since establishment will affect parasitism of the IAS *H. axyridis* resulting in low rates of parasitism at sites which have been colonised relatively recently by *H. axyridis*.

- The parasitism rate of the functionally-similar native ladybird *C. septempunctata* will be higher than for *H. axyridis*, concomitant with the predictions of the ERH.

We examined these hypotheses through a field survey of pupal parasitism (monitoring pupae of the two ladybird species for parasitism by the native parasitoids *P. fasciata, P. berolinensis* and *O. scaposus*, at a large spatial and temporal scale) and a semi-field experiment of adult parasitism (monitoring parasitism by the parasitoid wasp *D. coccinellae* in overwintering aggregations of adults of the two ladybird species within mesocosms).

Methods:

Field Survey - pupal parasitism:

Mature pupae of *C. septempunctata* and *H. axyridis* were collected between May and September from Loughborough, Leicestershire (during 2008-2011), Oxfordshire (2010-11), London (2010-11), Cambridge (2011) and Plymouth, Devon (2011) (Table 1). Pupae were collected by visually searching vegetation, primarily sycamore (*Acer pseudoplatanus* L.), lime (*Tilia* spp.) and nettle (*Urtica dioica* L.), between ground level and 2.5 metres and removing the leaf on which the pupae were attached. Collected pupae were kept in individual containers at a constant temperature and light regime (18°C, 16:8 L:D), and checked for emergence or parasitism on a daily basis. Parasitoids which emerged were allowed to reach adulthood and then identified after death. Owing to time constraints the few pupae from which nothing emerged were not dissected. Individual Phoridae from 66 *C. septempunctata* pupae and 32 *H. axyridis* pupae (representing half of the Phoridae broods) were dissected to determine species.

Semi-field survey - adult parasitism:

Both *H. axyridis* and *C. septempunctata* were collected from field sites in Crowmarsh Gifford, South Oxfordshire, in late September 2011. Native ladybirds at this time of year in Britain are still active but begin to move to overwintering sites. *Harmonia axyridis* has been observed to be active much later than native species in Britain and larval stages have been recorded in November (and exceptionally early December). As these individuals had been exposed to the possibility of parasitism in the field, they were monitored for parasitoid eclosion for a week in the laboratory, within a controlled environment (18°C, 16:8 L:D), before being placed outside in overwintering conditions. During the time spent within the laboratory the ladybirds were fed artificial diet, to ensure hydration, every other day (see Roy, Brown, Comont *et al.*, 2013 for details) and supplied with pea aphids (*Acyrthosiphon pisum* Harris) *ad libitum*.

Mesocosms were placed outdoors underneath Lombardy poplar trees (*Populus nigra* L. var. 'Italica') from the beginning of October 2011 (the usual time to begin overwintering) until mid-January 2012 (before the end of the winter dormancy period). Each individual mesocosm consisted of a black 14-litre bucket, with three holes drilled in the base to allow rainwater to drain. Mesocosms were filled with 2.5l of compost (John Innes No. 10), pressed down firmly and covered by 6.5l of fresh uncompressed leaf litter, collected from the area surrounding the mesocosms and frozen at -20°C for five days between collection and use in the mesocosms to kill any animals present. A 300x18x18mm L-shaped section of wood was placed on the leaf litter leaning against the side of the bucket. The top of the bucket was covered with dark green netting, with a mesh size (approximately 2 mm) small enough to prevent the ladybirds escaping, but large enough to allow *D. coccinellae* to enter.

The field-collected ladybirds were placed in the mesocosms so that each contained 40 adult ladybirds, either all *H. axyridis*, all *C. septempunctata*, or 20 of each of the two species. Twenty-four mesocosms (eight of each treatment) were located in Crowmarsh Gifford, Oxfordshire (10 km square SU68 in the UK Ordnance Survey grid system where the first *H. axyridis* was recorded in 2007), and a further 15 (5 of each treatment) were located at the Leeds University Farm, Tadcaster, West Yorkshire (10 km square SE44 in the UK Ordnance Survey grid system where the first *H. axyridis* was recorded in 2009). An additional fifty individuals of each species were kept individually in the laboratory and monitored for the duration of the experiment as a control, to test for pre-trial parasitism rates.

The mesocosms were collected in from the field in mid-January and the individual ladybirds were recovered. The number of individual ladybirds which had been parasitised by *D. coccinellae* was recorded at time of collection. All remaining ladybirds were monitored for *D. coccinellae* emergence over a period of six weeks. Owing to time constraints the individuals from which nothing emerged were not dissected.

Analysis:

Analysis was restricted to pupae from which either a ladybird or a parasitoid emerged. Parasitism rate was the proportion of these pupae which were parasitised.

For both datasets, the relationship between parasitism rate, host species, and years with *H. axyridis* presence was analysed using binomial General Linear Models (McCullagh & Nelder, 1989) in R version 2.15.2 (R Development Core Team, 2011), with full subsets model selection using Akaike's Information Criterion (AIC) values (Burnham & Anderson, 2002, Akaike, 1974).The model with the lowest AIC was chosen unless a model with fewer parameters was within 2 AIC units of this model, in which case the simpler model was preferred. The discriminatory ability of the models was evaluated using the Area Under the Receiver Operating Curve (AUC) (Fawcett, 2006).

For the pupal parasitism dataset, Generalized Linear Mixed Models (GLMMs) were constructed using the R package LME4 (Bates, Maechler & Bolker, 2011) and used to evaluate the relationship between parasitism rate and host species (*C. septempunctata* or *H. axyridis*), years of *H. axyridis* presence (1-6 years, mean 4.17 ± 1.19) and the interaction between host species and years of *H. axyridis* presence. The 10 km sq of collection was included as a random effect to take account of site-level effects not explained by the years of *H. axyridis* presence.

For the adult parasitism dataset, Generalized Linear Models (GLMs) were used to evaluate the per-mesocosm relationship between parasitism rate and host species (*C. septempunctata* or *H. axyridis*), treatment type (40 *C. septempunctata*, 40 *H. axyridis*, or a mixture of 20 *C. septempunctata and* 20 *H. axyridis*), and site (Oxfordshire or Yorkshire). Site was included as a fixed effect in this analysis, to test whether *H. axyridis* is parasitised at a lower rate in Yorkshire, where it had invaded more recently, than in Oxfordshire, having standardised the mesocosm environment as far as possible.

Results:

Field survey: pupal parasitism:

Overall, *H. axyridis* was parasitised at a much lower rate than *C. septempunctata*. Of the 4,595 live pupae collected (Table 1), 219 were parasitised (67/3868 *H. axyridis* (1.73%), 152/727 *C. septempunctata* (20.91%); see Table 2). Gregarious wasps (*O. scaposus*) emerged from one *H. axyridis* pupa and 21 of the *C. septempunctata* pupae, resulting in 423 individual wasps: 18 from the *H. axyridis* pupa and a mean 19.29 ± 7,79 (range 10-42) per *C. septempunctata* pupa.

Gregarious flies of the family Phoridae ("phorids") parasitised both *H. axyridis* (66 pupae parasitised, mean 3.44 ± 2.72 parasitoids per brood (range 1-15)) and *C. septempunctata* (131 pupae parasitised, mean 5.38 ± 3.15 parasitoids per brood (range 1-15)), producing 932 individual parasitoids. *A*ll 241 individuals (66 broods) examined from *C. septempunctata* were *P. fasciata,* as were 114 individuals (28 broods) from *H. axyridis,* while a further 10 individuals (4 broods) from *H. axyridis* were *P. berolinensis*. There was no evidence of hyperparasitism or cross-species multiparasitism in either host species.

Overall pupal parasitism rate was significantly lower in the IAS *H. axyridis* than in the native *C. septempunctata* (slope from *C. septempunctata* to *H. axyridis* -2.67 ± 0.18, $z = -15.23$, $p < 0.001$, AUC = 0.86). There was no significant effect on parasitism rate of the years of *H. axyridis* presence, or of the interaction between host species and years of *H. axyridis* presence (Table 3).

Semi-field survey: adult parasitism

Of the 1560 ladybirds originally placed in the mesocosms (780 of each species), 1475 were recovered (Table 4: 759 *C. septempunctata*, 716 *H. axyridis*) and the remaining 85 individuals were missing (presumably dead and decomposing, indeed elytra were found within the leaf litter). No successful parasitism was found in *H. axyridis*, but 43 (5.67%) *C. septempunctata* were successfully parasitised by *D. coccinellae*. There were no deaths from parasitism in the control samples, so the pre-experiment background parasitism rate was taken to be zero.

The best model to explain parasitism by *D. coccinellae* contained species and region (Table 5). Ladybirds were significantly more likely to be parasitised in Yorkshire than in Oxfordshire (Table 6: -2.63 ± 0.45, $z=-5.859$, $p <0.001$), and the native *C. septempunctata* was more likely to be parasitised than the IAS *H. axyridis*.Although not significant in the model (Table 6), inclusion of species as an explanatory variable improved overall model performance considerably (Table 5), and the model containing only 'species' was the best of the univariate models (Table 5), and was significant under the Kruskal-Wallis test ($X^2_1= 17.11$, p = <0.001). Parasitism rate did not differ significantly between the single- and mixed-species treatments for either ladybird species (Table 5).

Discussion:

Harmonia axyridis in its introduced range is parasitised at a considerably lower rate than either *H. axyridis* in its native range, or populations of *C. septempunctata* native to the introduced range of *H. axyridis*. Pupae of the invasive alien ladybird *H. axyridis* were parasitised at an exceptionally low level across Britain (1.73%) and adults were not found to be parasitized at all in our study. This is in contrast to the co-occurring native species *C. septempunctata,* which experienced reasonably high parasitism (20.91% pupae, 5.67% adults). This is consistent with the predictions of the ERH that enhanced performance of an IAS in the introduced range may result from a reduction or absence of natural enemies (Elton, 1958, Torchin, Lafferty & Kuris, 2001). The aposematic colouration and chemical defences of ladybirds causes them to suffer low rates of attack by non-specialist natural enemies (Roy *et al.*, 2013), and consequently this discrepancy in the rate of parasitism by specialist parasitoids is likely to represent an advantage for *H. axyridis*. However, further research is required to explore the population-level effects of this difference in parasitism rates.

Less than two percent of the *H. axyridis* pupae succumbed to parasitism by phorids in our study whereas *C. septempunctata* experienced high phorid parasitism rates (18 percent) at the same sites. It is also notable that parasitism of *H. axyridis* by phorids in Japan is much higher than in Britain; indeed nearly 15 percent of *H. axyridis* were parasitized by the native phorid *P. philaxyridis* found in Japan by Osawa (1992). However, phorid parasitism rates are known to vary considerably, for example phorid parasitism of *H. axyridis* across the native range was between 2% and 67% (Disney, 1997, Osawa, 1992, Park, Park, Hong *et al.*, 1996, Maeta, 1969). The absence of *H. axyridis* parasitism by *D. coccinellae* was notable in the studied overwintering aggregations particularly when considering that nearly six percent of the *C. septempunctata* were parasitized. It should be noted that the mesocosms did not represent ideal overwintering conditions particularly for *H. axyridis* which often overwinters in buildings or at elevated positions (Roy *et al.*, 2013, Nalepa, Kidd & Ahlstrom, 1996). However the high proportion of *H. axyridis* and *C. septempunctata* retrieved from the mesocosm is encouraging.

Pupal *H. axyridis* were found to be successfully parasitised by three species of parasitoid, one more than was recovered from *C. septempunctata*. Although the additional species, *P. berolinensis*, is known to parasitise *C. septempunctata* (Disney, Majerus & Walpole, 1994, Hodek *et al.*, 2012), in the study area it was only recovered from *H. axyridis* and two conifer-specialist ladybird species, *Aphidecta obliterata* L. and *Anatis ocellata* L. (R. Comont, unpublished data). This suggests that *H. axyridis* is susceptible to the suite of parasitoids in the system. The lack of evidence for increased rates of parasitism on *H. axyridis* over time from colonisation suggests that there is little or no adaptation of parasitoids to the arrival of *H. axyridis* so far. Despite the potential resource presented by the presence of high numbers of this large ladybird species, it is perhaps unsurprising that the parasitism rate is low given the short period of time since arrival of this IAS.

This limited adaptation to the presence of an IAS is consistent with results from other range-expanding or invasive taxa (Girardoz, Kenis & Quicke, 2006, Cornell & Hawkins, 1993, Menéndez, Gonzalez-Megias, Lewis *et al.*, 2008), including parasitism and inquilinism (in which an animal characteristically lives commensally in the nest, burrow, or dwelling place of an animal of another species) in the alien gall-wasp *Andricus quercuscalicis* (Burgsdorf), which has been monitored in Britain since its arrival in the late 1950s (Schönrogge, Stone & Crawley, 1996). Very low levels of parasitism/inquilinism (one species, <0.01 inquilines per gall) were recorded up to 30 years after establishment at sites in southern England, but after an additional five years, another 12 species were recorded from the gall and the wasp larva, and the mean number of inquilines per gall had risen to 0.26 (Schönrogge *et al.*, 1996). Even after around 55 years in the invaded range, however, parasitism of the wasp remained low (<10%) (Schönrogge *et al.*, 1996, Schönrogge, Stone & Crawley, 1995).

By contrast, rapid responses of natural enemies to invaders of some taxa have been observed. For instance, parasitism of the moth *Phyllonorycter leucographella* (Zeller) reached similar levels in the introduced range (Britain) compared to the native range (Turkey) approximately 20 years after establishment (Gröbler & Lewis, 2008). The ladybird *Olla v-nigrum* (Mulsant) was assimilated even more quickly into a native ecosystem. A native of North America and Oceania (Gordon, 1985), the species was introduced to New Caledonia in early 1987, quickly becoming widespread and abundant (Chazeau, Bouyé & Bonnet de Larbogne, 1991). Parasitism by the native phorid fly *Phalacrotophora quadrimaculata* Schmitz was first recorded in the wild in 1988, and by March 1989, two years after introduction, parasitism rates of 79% were reported from some populations (15-79%, mean 39%) (Disney & Chazeau, 1990).

Previous studies (Koyama *et al.*, 2008, Hoogendoorn *et al.*, 2002, Berkvens *et al.*, 2010) found that *D. coccinellae* showed no oviposition preferences between *H. axyridis* and *C. septempunctata* but successfully eclosed significantly less often from the IAS, suggesting the species might act as a sink for the parasitoid (Berkvens *et al.*, 2010, Hoogendoorn *et al.*, 2002). Other studies have found that *H. axyridis* produces chemicals that attract *D. coccinellae* (Durieux, Fischer, Brostaux *et al.*, 2012, Richerson & DeLoach, 1972, Al Abassi, Birkett, Pettersson *et al.*, 2001). We found no evidence that the presence of *H. axyridis* affected the rate of parasitism of *C. septempunctata* by *D. coccinellae*, and hence no support for a role for apparent competition. We did find a strong site effect for parasitism of adult ladybirds by *D. coccinellae* and it is possible that high rates of parasitism of native species may lead to parasitoid spillover into *H. axyridis* populations, if the resulting large numbers of parasitoids are unable to find many native hosts after depleting the populations. We only compared parasitism between *H. axyridis* and *C. septempunctata* and did not assess population-level effects on either host species. However, the low levels of parasitism found to date in *H. axyridis* populations suggests that the impacts of *H. axyridis* presence found by Roy *et al* (2012a) on common native ladybird species across Europe are likely to result entirely from direct interactions (predation and competition), rather than indirect interactions mediated by shared parasitoids.

Harmonia axyridis has aposematic colouration, strong morphological defences (spines) in the larval stage, and alkaloid-rich chemical defences at all life stages. Despite occasional observations of predation by birds, e.g. *Delichon urbicum* L. (R. Comont, personal observation) and mammals, e.g. *Rhinolophus ferrumequinum* (Shreber) (R. Comont, personal observation) and *Ursus arctos horribilis* Ord (Wang, Zhang & Zhang, 2007), predation is unlikely to have a regulatory effect on *H. axyridis* populations. Consequently, parasitism, which can reach 95% in populations of some species (Hodek *et al.*, 2012), is likely to be the dominant top-down factor regulating populations, and is effectively missing from *H. axyridis* populations in the introduced range at this early stage of invasion. The presence of several species of native parasitoid parasitising *H. axyridis* less than a decade after invasion does, however, suggest that the future recruitment of natural enemies to this abundant, albeit well-defended species is possible, particularly in the light of the documented time lags for recruitment of parasitoids to many invasive alien taxa (Cornell *et al.*, 1993, Schönrogge *et al.*, 1996, Schönrogge *et al.*, 1995, Girardoz *et al.*, 2006). Future research should focus on both the rate of parasitism and possible effects on the population dynamics of different species of ladybird. Indeed continued monitoring of *H. axyridis*, native species of ladybird and the parasites associated with them is essential to unravelling the web of interactions around this invasive alien species.

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Table 1. Number of pupae of the native ladybird *Coccinella septempunctata* and the invasive alien species *Harmonia axyridis* collected from sites given as 10km squares (10 x 10 km grid squares of the Ordinance Survey British National Grid) across England (Cambridge, Cambridgeshire; London, Middlesex; Loughborough, Leicestershire; various locations in south Oxfordshire; and Plymouth, Devon) between 2008 and 2011. Numbers in brackets indicate living pupae which produced either an adult ladybird or a parasitoid. 0 indicates no pupae found and – indicates site not monitored.

Site	**10km square**	***H. axyridis* arrival**	**2008**		**2009**		**2010**		**2011**	
			C. septempunctata	***H. axyridis***	***C. septempunctata***	***H. axyridis***	***C. septempunctata***	***H. axyridis***	***C. septempunctata***	***H. axyridis***
Cambridge, Cambridgeshire	TL36	2005	-	-	-	-	-	0	72 (72)	94 (94)
London, Middlesex	TQ27	2004	-	-	-	-	0	227 (212)	-	-
	TQ28	2005	-	-	-	-	0	342 (316)	0	311 (297)
	TQ39	2004	-	-	-	-	0	164 (141)	-	-
Loughborough, Leicestershire	SK51	2007	0	35 (35)	97 (92)	62 (58)	0	28 (28)	58 (58)	272(272)
	SK52	2007	0	63 (61)	31 (30)	191 (191)	0	230 (230)	73 (73)	581 (581)
Various locations, Oxfordshire	SP30	2007	-	-	-	-	19 (18)	21 (21)	-	-
	SP50	2006	-	-	-	-	-	-	0	9 (9)
	SU58	2009	-	-	-	-	14 (14)	279 (270)	-	-
	SU59	2007	-	-	-	-	0	165 (147)	3 (3)	331 (265)
	SU68	2007	-	-	-	-	38 (37)	197 (190)	171 (148)	446 (388)
	SU69	2008	-	-	-	-	189 (182)	3 (2)	-	-
Plymouth, Devon	SX45	2007	-	-	-	-	-	-	0	60 (60)

Table 2. Summary of the observations on emergence of *H. axyridis* and *C. septempunctata* pupae. Of the 4111 *H. axyridis* and 766 *C. septempunctata* pupae collected 3868 and 727 respectively produced either an adult ladybird or parasites. The number and percentage of adult ladybirds emerging or pupae successfully parasitized is provided. The parasites were identified as *O.scaposus* and Phoridae. The number and percentage of the hosts from which these parasites emerged is documented. The data is summarised across all collection sites and dates (2008-2011: Cambridge, Cambridgeshire; London, Middlesex; Loughborough, Leicestershire; various locations in south Oxfordshire; and Plymouth, Devon).

	Species of ladybird			
	Harmonia axyridis		*Coccinella septempunctata*	
	Number	%	Number	%
Adult ladybirds emerging	3801	98.27	575	79.09
Pupae successfully parasitized	67	1.73	152	20.91
Pupae parasitized by *Oomyzus scaposus*	1	0.03	21	2.89
Pupae parasitised by flies of family Phoridae	66	1.71	131	18.02

Table 3. Comparison of the GLMMs for the field survey of pupal parasitism by *Phalacrotophora fasciata*, *P. berolinensis* and *Oomyzus scaposus* on the IAS *H. axyridis* and native *C. septempunctata*. Δ AIC is calculated as the AIC of each model in turn minus that of the null model; lower AIC values indicate better-fitting models. The best model is highlighted in bold text.

Explanatory variables within the model	**AUC**	**AIC**	**Deviance**	**Model comparisons (to best model)**		
				Δ AIC	**Δ Deviance**	**p-value**
Species	**0.85**	**1319.7**	**1313.67**	**-**	**-**	**<0.001**
Species + years *H. axyridis* present	0.86	1321.2	1313.25	1.5	-0.42	<0.001
Species + years *H. axyridis* present + species*years *H. axyridis* present	0.86	1323.2	1313.25	2.5	-0.42	<0.001
Null (intercept-only)	0.78	1577.7	1573.71	258	260.04	1
Years *H. axyridis* present	0.77	1578.0	1572.03	258.3	258.36	0.20

Table 4. Number and percentage of *C. septempunctata* or *H. axyridis* parasitized by *D. coccinellae* from mesocosms, situated in Crowmarsh Gifford (Oxfordshire) or Leeds (Yorkshire), in which overwintering aggregations of single species (either 40 *C. septempunctata* or 40 *H. axyridis*) or both species (20 *C. septempunctata* and 20 *H. axyridis*) had been placed. n = total number of individuals recovered whereby in Oxfordshire there were a total of 320 individuals per treatment (24 mesocosms) and in Yorkshire there was a total of 200 individuals per treatment (15 mesocosms). The number of individuals recovered is lower than the total number of individuals originally placed in the mesocosms because a small proportion of individuals were not retrieved.

	Single species				**Both species**			
	C. septempunctata **(Oxfordshire n=317; Yorkshire n = 184)**		***H. axyridis*** **(Oxfordshire n=310; Yorkshire n = 179)**		***C. septempunctata*** **(Oxfordshire n=160; Yorkshire n = 88)**		***H. axyridis*** **(Oxfordshire n=145 Yorkshire n = 82)**	
	No. of *D. coccinellae*	**% parasitism**	**No. of *D. coccinellae***	**% parasitism**	**No. of *D. coccinellae***	**% parasitism**	**No. of *D. coccinellae***	**% parasitis**
Oxfordshire	**2**	**0.63**	**0**	**0**	**4**	**2.50**	**0**	**0**
Yorkshire	**28**	**15.22**	**0**	**0**	**9**	**9.18**	**0**	**0**

Table 5. Comparison table for GLMs of the semi-field survey of parasitism by *D. coccinellae* on overwintering aggregations of adult ladybirds (the native *C. septempunctata* and the IAS *H. axyridis*). Δ AIC is calculated as the AIC of each model in turn minus that of the null model; lower AIC values indicate better-fitting models. The best model is highlighted in bold text

Explanatory variables within the model	**AUC**	**AIC**	**Deviance**	**Model comparisons (to best model)**		
				ΔAIC	**Δ deviance**	**p-value**
Species, region	**0.52**	**78.1**	**37.53**	**-**	**-**	**<0.001**
Species, region, treatment	0.52	81.3	36.63	3.2	-0.9	<0.001
Region, treatment	0.52	94.4	51.75	16.3	14.22	<0.001
Species	0.51	128.9	90.30	50.8	52.77	<0.001
Region	0.53	130.0	91.36	51.9	53.83	<0.001
Species, treatment	0.52	132.5	89.91	54.4	52.38	<0.001
Treatment	0.52	143.6	102.99	65.5	65.46	<0.001
Null (intercept-only)	0.53	173.7	137.03	95.6	99.5	1

Table 6. Coefficients for each explanatory variable retained within the best model (species (*C. septempunctata* or *H. axyridis*) and region: Leeds (Yorkshire) or Crowmarsh Gifford (Oxfordshire). Individual significance levels (z-value and p-value) are also given.

Explanatory variable	**Coefficient (± SE)**	**z-value**	**p-value**
Intercept	-1.56 (±0.18)	-8.623	<0.001
Species	-19.77 (±1856.92)	-0.011	0.992
Region	-2.63 (±0.45)	-5.859	<0.001