Using artificial sentinel prey to quantify predation intensity under field conditions

Marco Ferrante

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Department of Agroecology, Science and Technology
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Department of Agroecology
Flakkebjerg Research Centre
Forsøgsvej 1
DK-4200 Slagelse
Denmark
Preface

This thesis is submitted to the Graduate School of Science and Technology (GSST) at the Faculty of Science and Technology, Aarhus University, Denmark to fulfil the requirement for obtaining the degree of Doctor in Philosophy. The research presented here was carried out between May 2013 and April 2017, at the Department of Agroecology, Flakkebjerg Research Centre, Aarhus University, Denmark; the research environmental change period (November 2015 – March 2016) was spent at the Entomology Section of the Estación Experimental Agropecuaria Manfredi, INTA, Córdoba, Argentina. The project was supervised by Gabor L Lövei (main supervisor) and Niels Holst (co-supervisor), and was funded by two EC-funded projects, the AMIGA (www.amigaproject.eu), and PURE (www.pure-ipm.eu), as well as Aarhus University. This thesis consists of five parts. The introduction presents the concept of ecosystem services and the need of developing monitoring tools for tracking the ecosystem services status. Particular attention is devoted to predation, which provides the ecological service of natural pest control. The second part includes a brief summary of the material and methods used during this research. The third part, results, consists of seven articles which have either been published (3) or submitted and under review (4). The fourth part includes a general discussion of the main findings of the articles prepared as part of this Thesis. The fifth part presents future perspectives and conclusions.
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This thesis is dedicated to all the people who have contributed to my education in one way or another during these years, also to those who are no longer with us.
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Summary

The overall aim of this PhD was to contribute to the expansion of ecological monitoring methods to assist the transition from the current practice of following structural changes in ecological communities to direct, functional methods in ecological monitoring. To achieve this, I worked on testing and further articulation of a sentinel prey method using artificial caterpillars made of plasticine in order to track the ecological interaction of predation, which is related to the beneficial ecosystem function of natural pest control. Artificial caterpillars are inexpensive and easy to use, and – unlike other types of sentinel prey - allow identifying predators responsible for the predation activity. Under field conditions, artificial caterpillars are attacked by both invertebrate and vertebrate predators, and can provide quantitative estimates, being eminently suitable to compare predation rates. Reviewing the published evidence of its use, and finding obvious and important imperfections in current practice, I made several recommendations to improve inter-study comparability. I collected evidence of predation intensity as influenced by urbanisation, by habitat manipulation to increase biocontrol, and by forest fragmentation, and tested its usefulness in post-release monitoring of transgenic crops. With the exception of predation in maize (11.7%d^{-1}), the predation rates found during this PhD project (23.9-59.5%d^{-1}) were higher than the published median predation rate on artificial caterpillars, indicating high potential predator pressure, and suggesting that top-down forces are important in ecological communities. Further field tests indicated higher predation pressure during night than day, and equivocal role of red vs. green caterpillar colouration. Laboratory tests indicated the ability of a generalist carabid to perceive chemical cues, but no preference for intact live prey over artificial caterpillars.
Sammendrag

De overordnede formål med dette ph.d.-projekt var at bidrage til udviklingen af nye overvågningsmetoder inden for økologi. De nuværende metoder anvender indirekte mål til at beskrive strukturelle ændringer i økologiske samfund, men nye overvågningsmetoder, som berører direkte måling af økologisk funktionelle sammenhænge, er påkrævet. Således arbejdede jeg med at udvikle og teste en metode baseret på ”sentinel prey” (udkigsbytte), som var kunstige sommerfuglelarver lavet af modellérvoks. Formålet med larverne var at måle prædationen, som er relateret til økosystemets naturlige bekæmpelse af skadedyr. Modellérvokslarver er billige at fremstille og nemme at anvende, og i modsætning til andre typer sentinel prey gør de det muligt at bestemme hvilke rovdyr der står bag prædationsaktiviteten. Under feltforhold angribes de kunstige larver af både hvirveldyr og hvirvelløse dyr. og de kan give kvantitative mål for prædationsintensiteten, hvilket gør det muligt at sammenligne de forskellige rovdys prædationsrate. Jeg gennemlæste eksisterende studier baseret på sentinel prey og fandt indlysende og vigtige mangler ved de nuværende metoder. På den baggrund lavede jeg flere anbefalinger til at forbedre muligheden for at sammenligne forskellige studier. Jeg målte prædationsintensiteten som den påvirkes af urbanisering, habitatændring til fremme af biologiske bekæmpelse, fragmentering af skovområder og testede endvidere metodens anvendelighed til monitoring af eventuelle sideeffekter af dyrkning af genmodificerede afgrøder. Med undtagelse af prædationsraten i majs (11.7% pr. dag) var prædationsraterne, som jeg fandt i dette ph.d.-projekt (23.9-59.5% pr. dag), højere end medianen af prædationsraten på kunstige larver rapporteret i litteraturen. Dette indikerer at top-down mekanismer er vigtige i økologiske samfund. Yderligere tests indikerede en højere prædationsrate om natten end om dagen samt en ukla effekt af om der blev anvendt røde eller grønne larver. Laboratorieforsøg med en løbebille, en generalist prædator, indikerede evnen til at opfange kemiske spor fra byttet, men alligevel foretrak den ikke det levende intakte bytte frem for de kunstige larver.
Introduction

The ongoing dramatic biodiversity loss now amounts to a new mass extinction, the sixth such event since the Palaeozoic era (Barnosky et al. 2011). While the first five were presumably caused by catastrophic abiotic events, today the threat is anthropogenic (Ceballos et al. 2015). Extinction is a natural process with a roughly constant “background” rate (Raup and Sepkoski 1982) that, together with speciation, determine the number of species (Ricklefs 2007) and their distribution in a region (Gaston 1998) over geological timescales. This background extinction rate is estimated to be 1-10 species $y^{-1}$ (Dirzo and Raven 2003), which for vertebrates corresponds to an average expected life of 1 million $y$ (Lövei 2017). Current estimates suggest that human activities increased the global extinction rate at least a thousand times above this rate (Hanski 2016; May 2010).

The impact of humankind on biodiversity has several forms, which are often consequences of interrelated activities, and population overgrowth. Habitat loss (Pimm and Raven 2000), agricultural intensification (Gliessman 2015), climatic change (Thomas et al. 2004), species invasions (Lövei 1997), and overharvesting (Novacek and Cleland 2001) are major threats to biodiversity. There are strong ethical arguments why biodiversity loss should be avoided (James 2015), but humankind also has a simple, pragmatic reason to preserve biodiversity. From a utilitarian point of view, several ecological processes produce benefits, named ecosystem services (ESs) (MEA 2005), upon which we depend for our survival.

Various ways of classifying ESs have been suggested (de Groot et al. 2002; Wallace 2007), and the Millennium Ecosystem Assessment (MEA) consolidated them into four classes: provisioning, supporting, regulating, and cultural services (MEA 2005). Further work indicated unresolved difficulties in distinguishing whether certain processes generate regulating or supporting ESs, thus these two were combined, and the recommended classification by the European Commission DG Environment groups ESs into three categories: provisioning, regulating and maintaining, and cultural services (Maes et al. 2013). These services are often impossible to replace, or it would be possible only at impractical costs (Daily 1997). Regrettably, the status of most ESs worsened in the last decade (MEA 2005; Carpenter et al. 2009).

Biodiversity is important for continued and resilient ecological functioning (Walker et al. 1999), and thus for the continued benefits in the form of ESs. Mace et al. (2012) identified two distinct views in regard to biodiversity and ESs. In one, biodiversity and ESs are almost synonymous, and this view assumes that preserving one ensures the health of the other. In the other, the two are independent, separate concepts. The nature of the relationship between biodiversity and
ecological processes is complex, and has been long debated. The various suggested models to describe this relationship include: the “idiosyncratic” (Lawton 1994), the “species richness-diversity” (MacArthur 1955), the “rivet” (Ehrlich et al. 1983), and the “drivers and passengers” (Walker 1992) concepts.

In spite of the ongoing debate, there is also consensus (Balvanera et al. 2006; Hooper et al. 2005). Empirical data suggest that a high number of species enables ecosystems to maintain their functionality even when one or few species disappear because of environmental or anthropogenic disturbance; which is usually referred as the “insurance” effect (Hooper et al. 2005). Understanding how biodiversity is linked to ecological functioning is a central problem in ecology (Peterson et al. 1998). Due to the biodiversity crisis, however, what has before been an important theoretical ecological problem, now gained new, practical, and possibly vital relevance for humankind.

Many anthropogenic activities affect biodiversity, ESs, or both, and in order to track their status, monitoring is needed. Monitoring can be done by comparing observed data with expected values (i.e. baselines), in order to track changes from what is considered “ordinary” (Hellawell 1991). Monitoring everything is impossible as well as impractical (Carignan and Villard 2002), and this is why monitoring is often mentioned together with the concept of bioindication (McGeoch 1998). The monitoring literature is vast but also chaotic. The basic elements are often missing or mixed up: what is the indicandum (the phenomenon to be indicated), what is an appropriate indicator (e.g. the group or organisms that will signal, indicate the phenomenon to be followed), and what is an appropriate index (the response that can/should be measured on the indicator) (Lövei 2014). There are only few examples discussing the importance of deciding what to monitor before launching a monitoring program (Regan et al. 2008). Although there is a general agreement that monitoring is essential, monitoring is not a “highly regarded scientific activity” (Spellerberg 2005).

Monitoring changes in biodiversity has a longer (even if chaotic) history than monitoring the status of ESs, and various methods for the former are available (e.g. changes in presence/absence, abundance, diversity, etc.). This led to a situation where ESs are also monitored by collecting data on the status and changes on species considered important for an ES, the so-called “ecosystem service providers” (Kontogianni et al. 2010). This however, amounts to monitoring ecological structures rather than the actual ecological processes on which ESs rely. This remains so even in the face of a few exceptions. In soil science, for example, monitoring ecological processes such as soil respiration or decomposition is easier by measuring the outcome than monitoring (or even identifying) the providers of these functions (Reed et al.
2005). There are only a few suggestions (Meyer et al. 2015) and even less experience in the use of these methods that can improve the quality of our monitoring the impact of various events, natural and anthropogenic, on the status of ecological processes whose outcome is so important for us.

**Predation**

Whether primary consumers within ecosystems are regulated by bottom-up or by top-down forces has been long debated (Faeth et al. 2005), and evidence exists for both (Power 1992). Nevertheless, it is evident that some form of limitation exists, as all primary consumers have the potential to overexploit their resources (Peterson 1999). Predators are ecologically very important, with the potential to control their prey (Hairston et al. 1960). In some cases, predators can become the “keystones”, holding ecological systems together, and maintaining their diversity, as in the classical experiments by the late Robert Paine (Paine 1966).

Primary consumers often exploit plant materials that we want for ourselves, and thus top-down control, in this context termed pest control, is an important ecological service. Biological pest control is classified among the regulating and supporting services (Maes et al. 2013), and the agents of natural pest control can be manifold, predators being prominent among them (Barbosa 1998).

Among arthropods, insects are the most common taxon in terms of both biomass and species richness (Gullan and Cranston 2009). Insect predators and parasitoids are important biocontrol agents (Jervis and Kidd 1996; Symondson et al. 2002), which have been successfully used for thousands years, with the oldest written record from China in 304 AD (Van Mele 2008). The economic value of biological control is substantial, even though such quantifications are riddled with methodological difficulties (Letourneau et al. 2015). For example, the value of the activity of natural pest control in a single crop is estimated at $84 million for the American states of Illinois, Indiana, Iowa, Michigan, and Minnesota in 2005 (Zhang and Swinton 2012), and the total value of pest control by insects in the U.S.A. is estimated at US$4.5 billion y⁻¹ (Losey and Vaughan 2006). Notwithstanding the uncertainties of valuation, there is solid evidence that improving habitat conditions for predators increases the intensity of biological control and predators are important in sustainable agriculture (Gurr et al. 2017).

Monitoring arthropod predation is complicated by the small size, and behaviour of the predators themselves. Recording changes in population density, or abundance, of one or a few predatory groups is likely to produce a distorted picture, because changes in predator densities cannot
directly be converted to predation intensity. Among different predator species, there is intraguild predation as well as cannibalism (Rosenheim et al. 1995), and predators can also be reduced by parasitoids. Consequently, a direct quantification of predation would be superior.

Predation can be observed directly in the field either by an observer (Kidd and Jervis 2005), or by using cameras (Grieshop et al. 2012). Although this method may provide reliable estimates of predation for sedentary predators (e.g. net spiders), it does not work equally well for vagile organisms (Kidd and Jervis 2005). The presence of the observer may be a disturbing factor for the predator, the prey, or both (Wade et al. 2005).

Prey can be marked with a molecular label (i.e. isotopic, or a radioactive element), and after a given time predators can be collected from the field and screened to detect the presence of the label (Kidd and Jervis 2005). Prey labelling is time consuming, and requires specific laboratory equipment, and training. Perhaps the most problematic issue is that the label may alter both the natural behaviour of the prey, and its attractiveness to the predator. Moreover, ensuring that the label does not spread via other mechanisms apart from predation (such as scavenging or biological secretions) is virtually impossible (Kidd and Jervis 2005).

Prey remains in the predator gut can be identified using morphological (Sunderland et al. 1995), chemical (Luck et al. 1988), serological (Greenstone 1996), or DNA-based (Symondson 2002) analyses, which can provide accurate information about predator-prey relationships. However, the main concern remains that these methods provide qualitative rather than quantitative indications of predation (Furlong 2015; Greenstone et al. 2014).

Several of the above mentioned disadvantages can be overcome if we use a monitoring method that is set up by the experimenter, the sentinel prey method. A known number of sentinel prey is left in the field for a given period (suggested time 24 h), after which the number of missing, or attacked prey is recorded, and the percentage of supposed predatory events can be calculated. Compared to others, the sentinel prey method allows controlling most variables (e.g. prey type and size, density, distribution, exposure time), and by identifying predators, the predation pressure can be allocated. The accuracy of this method depends on the precautions used to avoid prey escaping, calibrating other mortality factors, and reliably identifying predation signs (Lövei and Ferrante 2017). While the sentinel prey method does not provide an absolute measure of predation, it is suitable to compare predation rates (Howe et al. 2009; Lövei and Ferrante 2017), to evaluate the outcome of various management interventions, as well as for monitoring changes in space and time.

Sentinel prey can be real, or can be produced using an artificial material, like plasticine or dough (Seifert et al. 2016). Model prey have been used to quantify predation rates on snakes (Brodie III
1993), lizards (Daly et al. 2008), frogs (Saporito et al. 2007), and especially birds (Møller 1987). Even among real sentinels there can be considerable differences in physical and chemical qualities (Lövei and Ferrante 2017). However, such prey are frequently chosen for convenience but at the price of the monitoring soundness. For example, dead prey are often used (Lövei and Ferrante 2017), even if the information they provide concerns scavenging rather than predation (Furlong and Zalucki 2010). Sentinel prey arrangement, number of prey used, exposure time, and even attack categorisation vary widely, making the current practice unstandardised and thus comparisons between studies difficult (Lövei and Ferrante 2017).

Various authors raised doubts about how much the predation pressure detected by artificial prey resembles the real situation, for both vertebrates (Zanette 2002), and invertebrates (Remmel et al. 2011). Nevertheless, the use of an artificial prey allows manipulation, and thus has the potential of giving comparable results.

The most common artificial prey to measure arthropod predation is the “artificial caterpillar”, which resembles a lepidopteran larva (Howe et al. 2009). Artificial caterpillars are inexpensive, do not require rearing, are easy to produce and manipulate, and informative about predator identity, although at varying levels of resolution (Low et al. 2014). Since their first application (Edmunds and Dewhirst 1994), artificial caterpillars have been used in 26 countries of the world (Lövei and Ferrante 2017), although the method was described only 15 years after the first use (Howe et al. 2009). It is now recommended as the most suitable and practical quantitative method to assess arthropod predation (Meyer et al. 2015).

In this brief overview, specific needs were identified that determined the formulation of the aims of my work:

1. There is a need to develop and employ methods that can directly track /monitor ecological functions, especially those whose outcome leads to important ESs. One of these is predation, which importantly contributes to the ES of biological control.

2. Monitoring predation is riddled with complications. For easy, informative and cost-effective monitoring of predator activity and predation pressure, the artificial sentinel prey method seems suitable. However, there has been no synthesis of its existing, documented use, data were lacking especially from cultivated habitats, and various methodological aspects have been unclarified, hindering its widespread use, and leading to uncertainty of its suitability. Empirical evidence is needed to decide about the potential of the method.
My **aim** was to *examine the suitability* of the artificial sentinel prey method for monitoring the ecological function of predation in various situations, and thus to *expand the toolkit* of field ecology.

These aims were pursued via the following **objectives:**

1. *Synthesise the existing evidence* on the use of the sentinel prey, focused on invertebrate prey types (article I).

2. *Test the appropriateness* and usefulness of the artificial sentinel prey method in various settings and problems (articles II-V).

3. *Provide quantitative data* on attack rates on caterpillars in various countries, habitats and settings that indicate the probable predation pressure caterpillars may be exposed to, and how this pressure changes in relation to various habitat management factors (articles II-V).

4. I also wanted to contribute to the general *development of the sentinel prey method*, by investigating how selected physical (article VI) and chemical (article VII) characteristics of the sentinel prey may influence the predation rate, and clarifying whether these factors inflate the predation pressure or provide a conservative estimate compared to predation pressure on real prey.

The present PhD project was also contributing to two international projects, PURE (www.pure-ipm.eu) and AMIGA (www.amigaproject.eu). The overall aim of PURE was to increase the sustainability of agricultural production in Europe (i.e. reducing pesticide use), by developing new and improving existing Integrated Pest Management (IPM) methods. One of the important IPM strategies is conservation biological control, which seeks to create favourable conditions for native beneficial organisms that may increase the activity and the levels of biological control by natural enemies of various insect pests (Gurr et al. 2017). Within the PURE project, our work package activity was to test if using a specifically planted flowering seed mix into margins of wheat fields would increase the predation pressure. Field experiments over two years were done, and the results are included under objective #3, providing quantitative data on predation pressure in, and near various types of margins. The "Assessing and Monitoring the Impacts of Genetically modified plants (GMPs) on Agro-ecosystems" (AMIGA) project was an international collaboration between 22 partners in 15 European countries plus Argentina. The aim of the AMIGA project was to develop standardised protocols and monitoring tools to evaluate the effect of genetically modified plants growing outdoors (Arpaia et al. 2014). Most risk assessment evaluations are based on a few species in laboratory trials (Lövei et al. 2009), or selected groups of non-target organisms (Wraight et al. 2000), while the potential consequences on ESs are
understudied. One of the goals of AMIGA is to develop feasible monitoring methods to possibly detect unwanted, unforeseen environmental effects after-field release of transgenic plants as required by EU legislation (Gómez-Galera et al. 2012). My contribution to the project was investigating the usefulness of the artificial caterpillar method to monitor predation pressure in transgenic maize at ground and plant-canopy levels, and by collecting baseline data on predation rates by various predator groups in European and Argentinian maize fields. The outcomes are detailed under objectives #2 and 3.
Material and methods

Review of the sentinel prey method (article I)

The review of the sentinel prey method was done using elements of the systematic review. After identifying relevant publications, we used the collected material to test several formal hypotheses, and synthesised the state of art of the sentinel prey method.

Field experiments (articles II-VI)

During the course of this research, I used a total of 18,324 artificial caterpillars (15mm long and 3mm diameter, Fig.1a) made of non-drying green (and in some cases, red) plasticine (Denmark, Smeedi plus V. nr. 776609 and V. nr. 776620, respectively), and prepared using a modified garlic press (Fig.1b). Artificial caterpillars were glued to a piece of reed or bamboo, and placed at ground level emulating a real caterpillar moving on the ground, or climbing the plant stem (Fig.1c), or were otherwise pinned on plant leaves (Fig.1d). In the field experiments the artificial caterpillars had been exposed to predators for 24h. Because the sentinel prey was fixed, any predator trying to attack could not take them away, but left behind characteristic signs of the attack. Predation marks on artificial caterpillars were categorised into broad predatory groups using photographic records (Ferrante et al. 2014; Low et al. 2014).

Field work was carried out during the course of this PhD period, except for Ferrante et al. (2014, article II), for which field-collected data were already available. Data analysis, manuscript writing, revision and publication were all done during the duration of this PhD research.

For statistical evaluation, I used the R program (R Core Team, 2016.) and relevant packages available in that environment.

Laboratory experiment (article VII)

The tests were performed in the laboratories of Flakkebjerg Research Centre, Aarhus University. The experimental arena consisted in a plastic box (15cm x 15cm, 18cm high, Fig.2a, b) containing a layer of sterilised soil, and connected with a flexible tube to a glass Y-shaped olfactometer (4cm diameter, 20cm long main arm, two 18cm long “forks” at a 45° angle). At the end of the each fork, a Falcon tube containing the prey was attached using parafilm. Air flow was provided by a suction pump (KNF Neuberger, Trenton, NJ, USA, model NMP 830 KNDC), operated at 1000 cm³ min⁻¹, connected to the plastic box. Adults of Pterostichus melanarius were given 15 minutes to make a choice before ending each trial.
Fig. 1. The artificial caterpillar (a) used during this PhD research (2013-2016), produced using a modified garlic press (b) where instead of the mesh a coin (1 DK) was inserted. Artificial caterpillars were placed on the ground (c), or pinned on leaves (d). For further details, see article V. Photos: Marco Ferrante; Fig. 1b: Gabor Lövei.
Fig. 2. The experimental arena used for the choice tests (see Ferrante et al. submitted manuscript D, article VII). Photo: Marco Ferrante.
Results

Main findings

Objective 1. A review of the sentinel prey method was completed combining elements of a narrative and a systematic review (Lövei and Ferrante 2017, article I). Real sentinel prey were first used in 1976 (Speight and Lawton 1976), while the first use of artificial caterpillars is more recent (Edmunds and Dewhirst 1994). Real sentinels used were mostly lepidopteran eggs, exposed to predation in North American cultivated habitats; the detected median predation is 25.8% \(d^{-1}\) (range = 0.02–100\%d\(^{-1}\), \(n = 204\)). Artificial caterpillars were usually of green plasticine (median length = 25mm, median diameter = 3.5mm), used in temperate and tropical forests, but rarely elsewhere. Roughly half of the studies using artificial caterpillars (\(n = 18\)) focused only on bird predation, which is easily identifiable by beak marks (Low et al. 2014). The median predation rate on this type of sentinels was 8.8\%d\(^{-1}\) (range = 1.2–68.7\%d\(^{-1}\), \(n = 42\)).

Current use of the artificial caterpillars lacks standardisation, and several suggestions were made to amend imperfections. In particular, providing details on experimental design, running independent experiments by replacing all sentinel prey at the end of an exposure event, using an exposure time of 24h, and reporting all the predation marks found can considerably improve the comparability between studies (Lövei and Ferrante 2017, article I).

Objective 2. The artificial caterpillar method was successfully used in the following experiments:

2.1 An urbanisation study in Denmark (Ferrante et al. 2014, article II). In this experiment, predation pressure was quantified along a rural-urban gradient. Predation was higher (59.5\%d\(^{-1}\)) in the continuous forest than in the suburban (43.2\%d\(^{-1}\)) and urban (40.1\%d\(^{-1}\)) areas. The most frequent attacks were made by chewing insects, ants, and small mammals.

2.2. Testing the effect of establishing a flowering margin at wheat fields on predation pressure in Denmark (under the aegis of the PURE Project, Mansion-Vaquié et al. 2017, article III). Unexpectedly, predation rates in the non-manipulated grassy margin were higher than in the flowering margin. We found that chewing insect predation rate was positively related to the activity density of ground beetles ≥15mm, and we concluded that ground beetles may be important generalist predators in this habitat.

2.3. Evaluating the effect of various landscape factors on predation pressure in a maize-forest landscape in Argentina (Ferrante et al. submitted manuscript A, article IV). Invertebrate predation rates were significantly higher within the forest fragments and along the edges than within the crop, but the same was not true for vertebrate predation rates. Distance from a
continuous forest affected invertebrate predation rate positively and vertebrate predation rate negatively. Distance from the forest edge did not affect invertebrate predation rate within maize.

2.4. We compared predation pressure in GM vs. non-GM maize fields in Denmark, Slovakia, Romania, Italy, and Argentina within the AMIGA Project (Ferrante et al. submitted manuscript B, article V). Predation rate on the ground (15.7%d⁻¹) was significantly higher than in the maize canopy (6.0%d⁻¹). Ground predation rates were significantly higher in eastern (Romania 29.0%d⁻¹ and Slovakia 21.4%d⁻¹) than western (Denmark 12.8%d⁻¹ and Italy 13.2%d⁻¹) Europe, and significantly lower in Argentina (7.3%d⁻¹) than in Europe. Total predation rates were nowhere significantly different between GM (12.7%d⁻¹) vs. non-GM (11.1%d⁻¹) plots.

In conclusion, the method proved to be inexpensive and easy to use, which imply practical advantages over other methods, especially when laboratory facilities are not available. The greatest advantage is the ability to partition predation rates by different predatory guilds. During these experiments, a total of 17,104 artificial caterpillars were exposed to predators, and seven types of predators were identified attacking the artificial prey, most commonly chewing insects, ants, birds, and small mammals. Marks which are compatible with the radula of slugs were also found although slugs are non-predators (Ferrante et al. submitted manuscript C, article VI). Before the start of this work, six studies were available from cultivated fields; we now have such data from five additional locations.

Objective 3. The predation rates found during this PhD project (11.7-59.5%d⁻¹) were higher than the median predation rate on artificial caterpillars in the published literature (Lövei and Ferrante 2017), indicating high potential predator pressure. The relative contribution of different predatory groups varied greatly in the different settings. Attacks by chewing insects (excluding ants) were always the most frequent, ranging from 3.3-52.1%d⁻¹ of the exposed caterpillars. The lowest rates were found in maize fields in Romania, and the highest in a continuous beech (Fagus sylvatica) forest in Denmark near Sorø (Ferrante et al. 2014, article II). Ant attacks were never observed in winter wheat (Mansion-Vaquié et al., 2017, article III), or maize fields at Flakkebjerg (Ferrante et al. submitted manuscript B, article V), and ranged from 0.2-17.5%d⁻¹ in other habitats, with the lowest score in maize fields in Romania, and the highest in the heterogeneous maize-chaco serrano landscape in Argentina (Ferrante et al. submitted manuscript A, article IV). Bird attacks were extremely rare in maize in Argentina (0.13%d⁻¹), beech forest patches (0.43%d⁻¹) and winter wheat fields in Denmark (1.3%d⁻¹), but were frequent in the heterogeneous Argentinian maize-chaco serrano landscape (22.7%d⁻¹). Mammal attacks were never observed in small-plot maize in Argentina, and were rare in Italian (1.3%d⁻¹) and
Danish (1.8%d⁻¹) maize fields, but were frequent in patches of beach forest in Danish suburban areas (22.2%d⁻¹).

Objective 4. To contribute to the methodological development of the application of artificial caterpillars, I carried out field trials in Danish beech forest fragments to measure invertebrate and vertebrate predation rates on two different artificial caterpillar colourations (pairs of green-green, green-red, and red-red caterpillars) during day vs. night (Ferrante et al., submitted manuscript C, article VI). Predation rates at night (mean = 30.9%d⁻⁰.₅, SD = 46.3%d⁻⁰.₅, n = 330) were higher than during daytime (mean = 17.0%d⁻⁰.₅, SD = 37.6.₃%d⁻⁰.₅, n = 330). Total predation was higher on pairs of red (mean = 30.0 %d⁻¹, SD = 45.9%d⁻¹, n = 220) than green-red (mean = 22.3%d⁻¹, SD = 41.7%d⁻¹, n = 220), or pair of green (mean = 19.5%d⁻¹, SD = 39.7%d⁻¹, n = 220) caterpillars. Invertebrate predation was higher on red caterpillars (mean = 24.1%d⁻¹, SD = 42.9%d⁻¹, n = 220) than other prey, vertebrate predation rate did not significantly differ by prey colouration.

In another set of laboratory experiments, selection experiments were performed exposing the common generalist predator *Pterostichus melanarius* to various combinations of real vs. artificial prey (Ferrante et al., submitted manuscript D, article VII). Live prey were not preferred over artificial caterpillars by adults of this carabid, but dead caterpillars were more attractive than unhurt ones. Predators were also attracted to wounded and simulated wounded prey in the selection experiments.
The results of this PhD research are detailed in the following articles:


IV. **Ferrante M**, González E, Lövei GL, Predators do not spill over from forest fragments to maize fields in a landscape mosaic in central Argentina. Submitted to Ecology & Evolution, ECE-2017-04-00536


VI. **Ferrante M**, Barone G, Kiss M, Bozóné-Borbáth E, & Lövei GL. Predation on artificial caterpillars indicates no enemy-free time for lepidopteran larvae. Under review in Entomologia Experimentalis et Applicata, EEA-2017-0081

VII. **Ferrante M**, Barone G, Lövei GL. The carabid *Pterostichus melanarius* uses chemical cues for opportunistic predation and saprophagy but not for finding healthy prey. Under review in BioControl, BICO-D-17-00070
Article I

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REVIEW

A review of the sentinel prey method as a way of quantifying invertebrate predation under field conditions

Gábor L. Lövei* and Marco Ferrante*

Department of Agroecology, Flakkebjerg Research Centre, Aarhus University, Forsøgsvej 1, DK-4200 Slagelse, Denmark

Abstract  Sentinel prey can provide a direct, quantitative measure of predation under field conditions. Live sentinel prey provides more realistic data but rarely allows the partitioning of the total predation pressure; artificial prey is less natural but traces left by different predators are identifiable, making it suitable for comparative studies. We reviewed the available evidence of the use of both types of invertebrate sentinel prey. Fifty-seven papers used real prey, usually measuring predation on a focal (often pest) species, with studies overwhelmingly from North America. The median predation was 25.8% d⁻¹. Artificial sentinel prey (45 papers) were used in both temperate and tropical areas, placed more above ground than at ground level. The most commonly used artificial prey imitated a caterpillar. Up to 14 predator groups were identified, registering a median of 8.8% d⁻¹ predation; half the studies reported only bird predation. Predation on real prey was higher than on artificial ones, but invertebrate predation was not higher than vertebrate predation. Invertebrate but not vertebrate predation was negatively related to prey size. Predation near the Equator was not higher than at higher latitudes, nor in cultivated than noncultivated habitats. The use of sentinel prey is not yet standardised in terms of prey size, arrangement, exposure period or data reporting. Due to the simplicity and ease of use of the method, such standardisation may increase the usefulness of comparative studies, contributing to the understanding of the importance and level of predation in various habitats worldwide.

Key words  arthropods; artificial caterpillar; biological control; ecosystem service; mortality; top-down effects

Introduction

Predation is one of the important ecological interactions, and has substantial effects on the structure and stability of ecosystems (MacArthur, 1955; Hairston et al., 1960; Paine, 1966). Among arthropods, insects are the most important natural enemies in terms of biomass, abundance or diversity: an estimated 25% of insect species are predators or parasites in at least one life stage (Gullan & Cranston, 2009). In cultivated habitats, such interactions, especially the ones between pests and their natural enemies, is often economically important as well, providing substantial benefits. The natural enemies of the soybean aphid (Aphis glycines) provide an estimated US$84 million benefit in 5 mid-western U.S. states (Zhang & Swinton, 2012), and the economic value of the activity of natural enemies of arthropod pests in the U.S.A. was estimated at US$4.5 billion per year (Losey & Vaughan, 2006). A key question in such assessments is the reliability of the evaluation of the predators’ impact on their prey (Furlong & Zalucki, 2010; Zalucki et al., 2015).

Usually, the effect of predators on prey is evaluated by analysing correlative changes in the abundance of the interacting species or groups (Halaj & Wise, 2001). Nevertheless, for ecological understanding, a quantitative
assess the structure of the observed experiment. Direct evidence is usually difficult to obtain, especially when arthropod predators are involved, due to their small size, cryptic habits, and frequent night activity (van Alphen & Jervis, 2005). Predation usually happens very quickly, and predators consume their prey when or where is not possible to record the interaction, without leaving any evidence (Mills, 1997).

Methods to examine predation by invertebrates include field observations, prey labelling, and gut content identification using various techniques (Sunderland, 1987; Macfadyen et al., 2015), from morphological identification of prey fragments (Sunderland et al., 1995), to chemical evidence of feeding (sensu Luck et al., 1988), most frequently using serological (Greenstone, 1996) or DNA-based (Symondson, 2002) methods. Each one of these has several recognised limitations. Field observations do not work well for mobile species, may be biased by the presence of the observer (Wade et al., 2005), and are limited because the observer cannot be in several places at the same time (Kidd & Jervis, 2005). Prey labelling is very laborious, and the type of label may change the behaviour of the prey and/or the predator, distorting their natural relationship (Kidd & Jervis, 2005). Additionally, labelling is more practical when centres on a focal prey species, and less so for constructing a food web. Molecular techniques require special training and laboratory equipment, and can indicate prey–predator relationships with confidence and precision, but quantification is more problematic (Greenstone et al., 2014; Furlong, 2015).

An alternative method to study predation is to actively manipulate prey availability: experimentally establishing patches of prey (sentinel prey), and to record the rate of disappearance or traces of predation after a set period of prey exposure (Kidd & Jervis, 2005). While this method often includes “non-natural” elements, such as inflated prey densities, non-natural distribution, prey immobilisation, and these may distort the “real” predator–prey interaction (Kidd & Jervis, 2005), it is suitable for comparative purposes.

Such sentinel prey does not necessarily have to be real; a rather superficial similarity to real prey is often enough. Studies used artificial mimics of caterpillars (Howe et al., 2009), bird eggs (Moller, 1987), snakes (Brodie III, 1993), frogs (Saporito et al., 2007), lizards (Daly et al., 2008), and butterflies (Finkbeiner et al., 2012). Such artificial prey cannot move, defend themselves, or behave as true prey would, and the absence of chemical cues may conceal prey identity. In general, we do not understand how would-be predators perceive and process prey-derived information, especially factors that lead to an attack, and thus calibrating sentinel prey results to obtain “true” predation pressure using either type of sentinel prey is complicated. Some of the above factors increase, while others decrease attack rates.

Nevertheless, the use of artificial sentinel prey has several advantages over using real ones: it is cheaper, does not require prey rearing, producing them can be a simple and fast procedure (as in the case of caterpillars: Howe et al., 2009), and manipulating prey density and distribution is easy. Perhaps the most important advantage with respect to the use of real sentinel prey is that the predators can be identified by the marks left when attacking (although there are limits to the level of such identification, see Low et al., 2014b), which makes the partitioning of the total predation pressure possible. For these reasons, this method was suggested to obtain quantitative estimates of predation as an ecosystem service under field conditions (Meyer et al., 2015).

Real prey has been used in field experiments for a long time: the first uses to measure parasitism (Otake, 1967), or predation (Speight & Lawton, 1976) were published more than 40 years ago, while the first report on the use of artificial caterpillars is more recent (Edmunds & Dewhirst, 1994). Impacts of arthropod predation is frequently reviewed, including various methodological reviews (Greenstone, 1996; Symondson, 2002; Furlong, 2015; Macfadyen et al., 2015), but so far, there is no synthesis on the use of the sentinel method.

Our aim here is to present an up-to-date synthesis of the available quantitative evidence on predation pressure on invertebrates using the sentinel prey method. While we did not use the full protocol (see O’Connor et al., 2012), we applied several elements of the evidence-based synthesis to make our review more transparent and repeatable. After the analysis and presentation of the structure of the available evidence, we examined support for the following hypotheses:

1. Recorded predation pressure is higher on real than artificial prey. We expect this because real prey presents a wider and more realistic range of stimuli, making it more probable that predators find them, and upon finding, attack and consume them (Rilling et al., 1959).

2. Predation pressure exerted by invertebrates is higher than by vertebrates. We hypothesise this for two reasons. One is that there is a higher diversity and density of invertebrate than vertebrate predators (Gullan & Cranston, 2009), and the other one is that vertebrates probably process a wider range of stimuli before attack, and could be “turned off” more readily by non-natural prey stimuli.
(3) Predation pressure by invertebrates decreases with increasing prey size, while vertebrate predators attack bigger prey at a higher rate (Remmel et al., 2011). Prey can employ various tactics to defend themselves from predators, and a small potential prey is more vulnerable than a larger one. A small predator ought to be more prudent, and often should refrain from attacking larger prey due to the increased risk of sustaining an injury during attack that may incur fitness costs (Steffenson et al., 2014). No such aversion is expected from vertebrate predators, most of which are much bigger than their invertebrate prey considered in this review.

(4) There is higher predation pressure closer to the Equator than at higher latitudes. This would be a straight consequence of the species diversity gradient: species richness of predators is highest near the Equator, and is expected to decrease as one moves away from this region (Jeanne, 1979; Novotny et al., 2006).

(5) Predation pressure in general is lower in cultivated than non-cultivated habitats. Herbivores can be limited by food quantity or quality (bottom-up control) or by natural enemies (top-down control). In cultivated fields, frequent pesticide spraying can reduce herbivore numbers, plus decimate natural enemies that are generally more sensitive to pesticides than the target herbivores (Furlong et al., 2004). This may not be so in organic cultivation systems and under integrated pest management, where chemical treatments are less frequently used, leading to the higher importance of top-down control (Andow, 1991). In natural habitats, plant diversity is higher that leads to lower herbivore densities (Root, 1973), but natural enemies are not harmed by repeated human interventions. The combination of these factors makes it plausible to assume a higher predation pressure in natural than cultivated habitats.

We found that artificial prey are informative as well as real ones; they may underestimate predation intensity (although up to almost 80% of artificial prey can be attacked within 24 h). Sentinel prey does not suffer more vertebrate than invertebrate predation. Contrary to our hypotheses, predation does not seem to be higher near the Equator, and was not higher in cultivated habitats than natural ones. Invertebrate predators reacted to prey size as hypothesised. If properly standardised, the artificial caterpillar method is very promising for gaining a comparative understanding of the importance of predation in various habitats.

Materials and methods

Literature search

We searched the Web of Science (+BIOSIS + CABI) databases for articles reporting the use of sentinel prey (search performed on 2 June 2014) using the search terms “sentinel prey”, resulting in 36 854 hits. Search results were further refined by including only articles in the fields of agriculture, environmental sciences, ecology, entomology, biodiversity, conservation or forestry, leaving 2875 articles. These were searched for the occurrence of “predation” in the abstract, leaving 118 articles, and the abstracts of these articles were read for relevance. We also checked the reference list of these papers for further, possibly relevant papers. Relevant articles reported work in terrestrial habitats, and the sentinel prey had to be an invertebrate (any developmental stage). We included papers using both live and dead invertebrate sentinel prey. This process resulted in a final total of 57 articles. Articles publishing results of experiments with artificial caterpillars (excluding indoor trials: Remmel & Tammaru, 2011; Skelhorn et al., 2014) were collected through a similar search (performed on 6 August 2015), using the terms “artificial caterpillar AND predat*”, and “predat* AND plasticine”. This resulted in 86 and 123 hits, respectively. Of these, 26 were relevant articles. Fifteen additional articles were found via references in the articles identified by the search, and 3 unpublished manuscripts were obtained from correspondence with the authors. Due to the great similarity in the methodology and the use of the artificial prey, we included the paper by Church et al. (1997). In total, we extracted data from 101 articles reporting experimental results using real or artificial invertebrate prey under field conditions.

Data extraction

The following data were extracted from the identified articles:

(1) location (biogeographical region, continent, country, latitude), habitat type (natural, cultivated or urban), and habitat detail (information on crop, habitat management);

(2) particulars of the sentinel prey: in the case of real prey, its status (dead or alive), taxonomical category, and developmental stage. For artificial prey, we extracted information on coloration, material, and size (length and diameter);
(3) experimental setup: information on the experimental setup included the treatments, how prey were secured (e.g. glue, pins, thread), prey position (on ground or above ground), height above ground, the number of prey models used per treatment, the total number of prey exposed to predation, inspection frequency, and exposure time;

(4) predators identified: under these columns, we sought to extract the most detailed information presented, but also combined them into higher categories. This inevitably resulted in mixed categories, some of which were taxonomic at various levels (e.g. birds, arthropods, ants), others bionomic (chewing insects, parasitoids).

Predation was a measure of disappearance or mortality (for real prey) or the number of prey attacked (for both real and artificial prey). In articles using real prey, data were mostly presented as percentage or proportion of prey attacked ($n = 44$ articles), with both percentage and number of prey attacked ($n = 5$), only numbers attacked ($n = 5$), or mean number of prey attacked ($n = 3$). Data collected using artificial caterpillars were mostly presented as number of prey attacked ($n = 16$), fewer used percentage or proportion of prey attacked ($n = 11$), or published only means ($n = 3$).

Predation rates measured were reported as in the text, or where possible, were standardised to attack rates over 24 h exposure time (e.g. where predation over 48 h was reported, we divided it by 2 to obtain daily predation rates). Predation rates from experiments using an exposure time $<24$ h were entered in their original form but excluded from statistical analysis.

We examined the relationship between prey size and predation rates, as well as predation rates and latitude, separately for total predation pressure, and for selected groups. Predation pressure data were expressed as percent prey attacked over 24 h ($% \text{ predation} \cdot d^{-1}$). No transformation was able to normalise these data, so for all the analyses, we used the Kruskal–Wallis non-parametric test. Analyses and graphical presentation were performed in R 3.2.2 software (R Core Team, 2015).

Results

We found 57 papers using real sentinel prey, and 45 on artificial caterpillars, of which 42 were field trials, and 3 articles (Howe et al., 2009; Barber, 2012; Low et al., 2014b) dealt with methodological aspects. The oldest paper using real prey (Speight & Lawton, 1976) precedes the use of artificial caterpillars (Edmunds & Dewhirst, 1994) by nearly 20 years. The use of both types of sentinel prey gradually increased. After a single paper between 1976 and 1985, 5 were published between 1986 and 1995, 15 between 1996 and 2005, and 36 between 2006 and 2015. Four articles using artificial caterpillars were published between 1994 and 2005, and the remaining 41 in the period 2006–2015.

Biome, country, and habitat

Articles using real prey report work mostly done in the temperate region ($n = 51$), with a prevalence for North America ($n = 42$). There were only 5 studies in the tropics (Jones et al., 2001; Pearce & Zalucki, 2006; Kaufman & Wright, 2009; de Medeiros et al., 2011; Vieira et al., 2014). There was also a bias for cultivated habitats and farms ($n = 51$), with fewer studies in forested (Duan et al., 2011; Jennings et al., 2014), urban (Dobbs & Potter, 2014), grassland (Lövei & Brown, 1993), or wetland (Wiebe & Obrycki, 2004) habitats. In one case (Kaufman & Wright, 2009), the habitat chosen for the experiment was not clearly stated.

Studies using artificial caterpillars were geographically more evenly spread, with less difference between temperate (22 studies from 13 countries), and tropical (19 studies in 13 countries) areas. Most ($n = 15$) studies were done in Europe (Denmark, Finland, France, Hungary, Ireland, Spain, and the United Kingdom), followed by South America ($n = 8$), Australasia, Africa ($n = 5$ each), Asia, Central America, and North America ($n = 4$ each). Two papers (Church et al., 1997; Speed et al., 2005) compared two countries on the same continent; and Barbaro et al. (2014) compared predation pressure on two continents. In both temperate and tropical areas, artificial caterpillars were generally used in forested habitats ($n = 17$ and $n = 14$, respectively), but urban ($n = 5$ and $n = 1$, respectively), or cultivated areas ($n = 2$ and $n = 4$, respectively) were also studied.

Prey types used

More experiments used live ($n = 59$) than dead ($n = 14$) real prey; only 2 studies (Lövei & Brown, 1993; Chen & Ruberson, 2008) used both. In a few cases (Wilson et al., 2004; Mathews et al., 2011; Smith & Gardiner, 2013), the status of the sentinel prey was unclear. The most common prey used were Lepidoptera ($n = 43$, 18 species), Coleoptera ($n = 17$, 10 species), Hemiptera ($n = 15$, 6 species), and Diptera ($n = 5$, 4 species). Eggs were the most frequently used life stage ($n = 52$), followed by larvae ($n = 14$), pupae or nymphs ($n = 11$).
only 3 studies (Brown & Mathews, 2008; Winqvist et al., 2011; Ximenez-Embun et al., 2014) used adults. Sentinels were mostly immobilised (n = 10) or naturally immobile (n = 62), but not always (n = 8), with 6 experiments (Mathews et al., 2004; Wiebe & Obyrcky, 2004; Chen & Ruberson, 2008; Hong et al., 2011; Mathews et al., 2011; Lee & Edwards, 2012) using mobile and immobile prey simultaneously.

Artificial caterpillars were made of either plasticine (n = 33), or dough (a mixture of flour, water, lard, and colourant, n = 11). Green (n = 30) or brown (n = 6) were common colour choices, but they could also have a green body with yellow (Loiselle & Farji-Brener, 2002), or brown “head” (Faveri et al., 2008; Ruiz-Guerra et al., 2012), or green body with eyespots (Hossie & Sherratt, 2012; 2013). Suzuki & Sakurai (2015) used artificial caterpillars designed to mimic bird droppings (using larvae of Macrauza maxima and Apochima juglanaria as models). However, in most cases (n = 23) the artificial sentinel prey was “a general caterpillar”, with no indication of any species to be imitated. The typical size used (median diameter = 3.5 mm, median length = 25 mm, n = 34 and n = 39, respectively) was close to the median size of real caterpillars (Novotny & Basset, 1999). Information was occasionally missing on the colour (González-Gómez et al., 2006; Lluch et al., 2009; Moreno & Ferro, 2012; Poch & Simonetti, 2013), diameter (González-Gómez et al., 2006; Skoczylas et al., 2007; Lluch et al., 2009; Poch & Simonetti, 2013; Molleman & Safian, 2015; Molleman et al., 2016), or length (González-Gómez et al., 2006; Lluch et al., 2009) of the artificial caterpillars used.

Sample arrangement and size

Real prey was placed mostly above-ground (median height = 1.1 m, range = 0.15–2.68 m, n = 33) in preference to ground level (n = 17), with a single study (Lee & Edwards, 2012) using below-ground prey. In 27 articles, the height from the ground was not specified. The majority (n = 35) of the sentinel prey were secured onto various substrates (cardboard, wax paper, plastic, mesh cloth). Prey was also secured using pins (Naranjo, 2005; Hong et al., 2011; Lundgren & Fergen, 2011; Mathews et al., 2011; Dobbs & Potter, 2014), staples (Wilson et al., 2004; Weber et al., 2008; Olson & Ruberson, 2012), thread or wire (Lövei & Brown, 1993; Ehler, 2002, 2004, 2007; Mathews et al., 2004; Takeuchi & Watanabe, 2006). In 3 studies (Lövei & Brown, 1993; Naranjo, 2005; Hong et al., 2011), prey was placed on the soil surface unsecured. The method used to expose the prey was not specified in 5 cases (Wiebe & Obyrcky, 2004; de Medeiros et al., 2011; Mathews et al., 2011; Bickerton & Hamilton, 2012; Sigsgaard, 2014).

Artificial caterpillars have also been mostly placed above ground (median height = 1.5 m, range = 0.2–5 m, n = 36); with 6 using ground exposure, and a single study (Posa et al., 2007) both. In 8 studies (22.2%), the height was not specified. Prey was glued (n = 15), fixed by threads/wires (n = 10), pins (n = 8), or simply placed on a ground or a board (n = 8). Three papers (Loiselle & Farji-Brener, 2002; Faveri et al., 2008; Seifert et al., 2015) did not report the prey placement method.

The distance between individual sentinels was usually justified by qualitative considerations, such as the ecology of the predatory guild investigated rather than on a quantitative method (e.g. test for autocorrelation, or variogram analysis). Even with real prey, the density chosen often seemed arbitrary, and was rarely justified.

The number of prey items per treatment did not differ significantly pre prey type (real vs. artificial prey: median = 541.6 (range = 10–24 000, n = 60) vs. median = 216 (range = 7–7200, n = 46), Kruskal–Wallis nonparametric test, P = 0.062) but total number of prey per experiment was significantly higher when using real prey (real vs. artificial prey: 2240 (range = 28–340 200, n = 77) vs. 658.5 (range = 32–14 400, n = 46), Kruskal–Wallis nonparametric test, P = 0.002). Egg density (median = 20, range = 5.4–1000, n = 38) is lower than the number of egg clusters used per treatment (median = 80, range = 2–800, n = 38). However, sample sizes claimed are not always true replicates. For example, reports of 1000 eggs (Rueda & Axtell, 1997) cannot be considered independent prey; in those cases, the number of eggs clusters would represent true sample size.

Exposure times varied widely. Real prey have been exposed for 24 h (n = 21), 48 h (n = 19), or 72 h (n = 12), with a median = 48 h, ranging from 15 min (Diaz et al., 2004; Mathews et al., 2004) to 672 h (Duan et al., 2011). In most cases (n = 66) prey was only checked once, at the end of the (single) exposure period.

Artificial caterpillars have been mostly exposed for 24 h (median = 72 h), ranging from 7 h (Edmunds & Dewhirst, 1994; Suzuki & Sakurai, 2015) to 1512 h (Lemessa et al., 2015). Generally, when short exposure time was used, all caterpillars (attacked or not) were collected, and a new trial was started (inspection time = exposure time, n = 26), while in cases of long exposure, predation was periodically assessed, and only missing or attacked caterpillars were replaced (n = 7). Five studies used a variable exposure time, depending on attack rates. For example, Rowland et al. (2008) ended their trial when 50–60% of the sentinel prey were attacked. One study (Ruiz-Guerra et al., 2012) did not publish the exposure time.
Together with the quantification of predation level, most studies \((n = 36)\) carried out parallel arthropod sampling (pitfall trapping, sticky traps, sweep net, or visual sampling). A single study (Grieshop et al., 2011) used 3 species of real sentinels, *Acrobasis vaccinii*, *Galleria mellonella*, and *Popillia japonica*, for testing a video system to record insect predation.

**Predators identified**

While parasitoids were the only natural enemy group readily identifiable using real prey, identified natural enemies of artificial caterpillars \((\text{median} = 3 \text{ per study})\) included vertebrates \((\text{birds} n = 40; \text{mammals} n = 14; \text{reptiles} (Posa et al., 2007), \text{as well as} \text{invertebrates, among} \text{them ants} (n = 12), \text{predatory wasps} (Faveri et al., 2008; Jordani et al., 2015; Molleman & Safian 2015; Sam et al., 2015a,b), \text{parasitoid wasps} (Tvrdikova & Novotny, 2012; Howe et al., 2015), \text{other insects, predatory bugs, cockroaches} (Molleman et al., 2016), \text{and spiders} (Posa et al., 2007).

**Guilds investigated**

Real prey has mostly been used to evaluate the impact of a particular predator/parasitoid, including the ants *Pheidole megacephala* (Jones et al., 2001), and *Solenopsis invicta* (Diaz et al., 2004; Olson & Ruberson, 2012), the encyrtid wasp *Oobius agrili*, a parasitoid of the invasive emerald ash borer (*Agrilus planipennis*) (Duan et al., 2011), the egg parasitoid *Trichogramma brassicae* (Burgio & Maini, 1995), the pupal parasites of *Musca domestica* (Mullens et al., 1986), and telenominaeid parasitoids of *Euschistus heros* (Vieira et al., 2014). The importance of predation and parasitism on a specific prey, usually a pest, was studied on the lepidopteran pests *Cydia pomonella* (Libourel & Franck, 2012), *Spodoptera exigua* (Ehler, 2004; 2007), and *Udea stellata* (Kauffman & Wright, 2009), as well as the stink bugs *Euschistus servus* (Tillman, 2010), and *Nezara viridula* (Ehler, 2002; Tillman, 2010). The method was used to examine intraguild predation in ladybirds (Gardiner et al., 2011; Smith & Gardiner, 2013), and the antagonistic relationship between ants and the parasitoid *Trichogramma minutum* (Mathews et al., 2011).

Studies using real prey focused on one or more taxa \((n = 10)\), or guilds (e.g. nocturnal predators, chewing predators, \(n = 13\)), but more often \((n = 40)\) these studies were not able to identify the predators responsible for prey mortality. A minority of the experiments used exclusion to apportion predation to certain groups, for example, by excluding birds (but not small mammals) (Lövei & Brown, 1993); or vertebrates (Meehan et al., 2012; Dobbs & Potter, 2014).

About half of the studies using artificial caterpillars focused on several groups of predator \((n = 20\); Table S1\), the rest \((n = 18, \text{Table S2})\) looked only on predation by birds. Three studies (Bereczki et al., 2014; 2015; Maas et al., 2015), while focusing on bird predation, also presented attack rates by other predators. Generally, reported total predation was accompanied by data on the relative contribution of each identified predatory group, but in 4 papers (Koh & Menge, 2006; Richards & Coley, 2007; Faveri et al., 2008; Moreno & Ferro, 2012) this information was missing.

**Predation rates**

Predation rates measured using real prey had a median value of 25.8% \(d^{-1}\) \((range = 0.02–100\% d^{-1}, n = 204)\), significantly higher \((\text{Kruskal–Wallis nonparametric test}, \chi^2 = 9.3, \text{df} = 1, P < 0.05)\) than predation on artificial caterpillars \((\text{median} = 8.8\% d^{-1}, \text{range} = 1.2–68.7\% d^{-1}, n = 42)\). Invertebrate predation on real prey \((excluding vertebrates)\) was much lower, with a median \(3.4\% d^{-1}\) \((range = 1.6–22.6\% d^{-1}, n = 16)\).

Of artificial prey, those made of dough were predated significantly more than clay ones \((Sam et al., 2015b; Molleman et al., 2016), and prey dispersion had no effect on predation \((Church et al., 1997)\). Invertebrate predation rates on artificial prey were higher at ground level \((\text{median} = 32.8\% d^{-1}, \text{range} = 0–58.3\% d^{-1}, n = 9)\) than above ground \((\text{median} = 2.9\% d^{-1}, \text{range} = 0.88–65.8\% d^{-1}, n = 29)\) but only marginally significantly \((\text{Kruskal–Wallis non-parametric test}, \chi^2 = 3.76, \text{df} = 1, P = 0.05)\).

A positive correlation was found between predation rate on real prey and the abundance of ground beetles \((Speight & Lawton, 1976; Menalled et al., 1999; Prasifka et al., 2006)\), ground-active predators in general \((O’Neal et al., 2005)\), or predatory insects \((Chang & Snyder, 2004)\) but in some cases, this was not so \((Pearce & Zalucki, 2006; Lee & Edwards, 2012)\).

Predation on artificial caterpillars and bird densities in Hungarian forests \((Bereczki et al., 2014)\), and predation and ground beetle abundance in cultivated fields in Denmark \((Mansion-Vaquie et al., unpublished)\) were positively correlated.

Experiments with sentinel real prey generally supported the positive role of habitat complexity on predator impact in lawns \((Dobbs & Potter, 2014)\), the similarly positive effect of floral diversity \((Werling et al., 2011; Sigsgaard, 2014 but see Mansion-Vaquie unpublished)\), intercropping \((Prasifka et al., 2006)\), and landscape...
complexity (Winqvist et al., 2011). Predation along a grassland successional gradient, using real sentinel prey, the wine weevil (Otiorrhynchus sulcatus) eggs and blowfly (Lucilia sericata) larvae, showed high predation rates (up to 80% d\(^{-1}\)) in the very early (1 year) and the late (16 years) stages of secondary succession (Lövei & Brown, 1993).

Predation on artificial caterpillars can be negatively (Faveri et al., 2008) or positively (Jordani et al., 2015) related to habitat area. Habitat complexity was positively related with predation on artificial caterpillars in Chilean forests (Poch & Simonetti, 2013), negatively related in Brazilian cerrado (Moreno & Ferro, 2012), and had no significant effect in Hungarian oak forests (Bereczki et al., 2014).

Fragmentation usually increased predation pressure, both in temperate (González-Gómez et al., 2006; Lluch et al., 2009; Barbaro et al., 2012) and tropical (Ruiz-Guerra et al., 2012) habitats, but in a transcontinental experiment performed in France and New Zealand, intermediate levels of forest fragmentation increased avian insectivory (Barbaro et al., 2014). In Panama, neither fragmentation nor distance from the edge affected predation pressure (Koh & Menge, 2006). Bird predation usually increased at the edge (Scoczylas et al., 2007; Barbaro et al., 2014; Maas et al., 2015), although inconsistent trends were also registered (Bereczki et al., 2015).

In forested habitats, predation rates by both invertebrate and vertebrate predators increased from the Equator toward the poles (Figs. 1A and B), although the relationship was not significant. Predation was higher on trees damaged by herbivory (Mäntylä et al., 2008, 2014; Sam et al., 2015a), and this can be linked to the herbivore-induced plant volatile cineole (Low et al., 2014a), but not to methyl jasmonate (Mäntylä et al., 2014).

Predation on artificial caterpillars is higher in the canopy (Loiselle & Farji-Brener, 2002), or in tree-fall gaps (Richards & Coley, 2007) than in the tropical understory, and higher in tree-fall gaps than in the canopy (Seifert et al., 2016). Predation was higher during the day than at night in the Guianan rainforest (Seifert et al., 2016), increases with tree species richness at small but not large spatial scales (Muiruri et al., 2015); and decreases with increasing elevation (Sam et al., 2015a). Anthropogenic disturbance increases predation rates in forests in the Philippines (Posa et al., 2007) and Costa Rica (Seifert et al., 2015), but decreases in temperate forest fragments in Denmark (Ferrante et al., 2014).

Vertebrate predation on artificial caterpillars was higher (median = 3.9% d\(^{-1}\), range = 0–22.2% d\(^{-1}\), \(n = 61\)) than invertebrate predation (median = 3.3% d\(^{-1}\), range = 0–65.8% d\(^{-1}\), \(n = 38\)) but not significantly so (Kruskal–Wallis nonparametric test, \(\chi^2 = 0.32, df = 1, P = 0.41\)). We found a negative linear relationship between the length of the artificial prey and arthropod predation rate \((P = 0.002, adj. R^2 = 0.22, Fig. 2A)\), but none such exists for vertebrates \((P = 0.546, adj. R^2 = 0, Fig. 2B)\). Excluding social insects did not change the results.

Invertebrate predation was higher in temperate (median = 5.0% d\(^{-1}\), range = 0.94–58.3% d\(^{-1}\), \(n = 15\)) than tropical forest (median = 4.1% d\(^{-1}\), range = 0–65.8% d\(^{-1}\), \(n = 7\)) or cultivated areas (median = 3.1% d\(^{-1}\), range = 0.94–58.3% d\(^{-1}\), \(n = 15\)) but there was no significant difference between habitats (Kruskal–Wallis nonparametric test, df = 1, \(P > 0.05\) for all possible comparisons).

Vertebrate predation was higher in tropical (median = 6.7% d\(^{-1}\), range = 0–13.3% d\(^{-1}\), \(n = 14\)) than temperate forests (median = 5.5% d\(^{-1}\), range = 0–22.2% d\(^{-1}\), \(n = 30\)), and crop areas (median = 1% d\(^{-1}\), range = 0–10.7% d\(^{-1}\), \(n = 15\)). Vertebrate predation in cultivated areas was significantly lower than in temperate (Kruskal–Wallis nonparametric test, \(\chi^2 = 6.52, df = 1, P = 0.011\)) or tropical (Kruskal–Wallis nonparametric test, \(\chi^2 = 5.47, df = 1, P = 0.019\)) forests.

Predation pressure in noncrop habitats adjacent to cotton fields in Uganda is higher than in the fields themselves (Howe et al., 2015). The presence of forest areas near oil palm in Malaysian Borneo (Gray & Lewis, 2014) and cacao plantations in Indonesia (Maas et al., 2015) increase arthropod and bird predation, respectively. In Ethiopian urban gardens, both invertebrate and vertebrate predation rates are higher in tree-poor (1.85% d\(^{-1}\) and 1.46% d\(^{-1}\), respectively) than tree-rich (1.44% d\(^{-1}\) and 1.40% d\(^{-1}\), respectively) landscapes.

Experiments with real sentinel prey indicated that pesticide use reduces parasitism and predation in orchards (Atanassov et al., 2003, Monteiro et al., 2013), in maize and perennial grassland (Meehan et al., 2012) but has no negative impact in turf (Larson et al., 2012). Non-harvested plots boost predation which decreases with distance from such areas (Hossain et al., 2012), and fertilization in cotton reduces predation but not parasitism (Chen & Ruberson, 2008). A study in transgenic Bt and non-Bt cotton found no difference in predation on eggs and pupae of Pectinophora gossypiella (Naranjo, 2005), but predation on Nezara viridula eggs is higher in Bt- than non-Bt cotton (Olson & Ruberson, 2012).

Other factors influencing predation

Countershading (Edmunds & Dewhirst, 1994; Rowland et al., 2007), or reverse countershading (Rowland et al.,
Fig. 1 The relationship between geographical latitude (expressed in degrees) and predation rates measured using artificial caterpillars by invertebrate (A) and vertebrate (B) predators in forested habitats. The solid lines represent the fit of a linear regression, dashed lines indicate ± SE. Regression equations: (A) $y = 6.1517 + 0.2275x$, $P = 0.341$, adj. $R^2 = -0.002274$; (B) $y = 4.6613 + 0.0693x$, $P = 0.215$, adj. $R^2 = 0.01377$.

Fig. 2 The relationship between the length of artificial caterpillar sentinel prey and predation by invertebrate (A) and vertebrate (B) predators. The solid lines represent the fit of a linear regression, dashed lines indicate ± SE. Regression equations: (A) $y = 33.618 - 0.951x$, $P = 0.002$, adj. $R^2 = 0.2158$; (B) $y = 3.6831 + 0.0650x$, $P = 0.546$, adj. $R^2 = -0.01357$.

2008) can reduce bird predation, although not in all cases (Speed et al., 2005; Hossie & Sherratt, 2012). The defensive value of eyespots remains unclear, as it can be effective in itself (Hossie & Sherratt, 2013) or when interacting with countershading (Hossie & Sherratt, 2012), but it can also cause increased mortality (Hossie et al., 2015). Mimicking bird dropping can significantly reduce bird predation only when interacting with prey.
potential attractiveness regardless of its shape (Buasso et al., 2015b) suggests that plasticine may be non-predators (i.e., insects, non-predatory small rodents) never been studied. A nonspecified amount of marks by sons why a predator should attack an artificial prey have testing hypotheses and doing comparative studies. Few age is the ease of manipulation, making them ideal for vertebrate and invertebrate predation rates on artificial caterpillars are as informative as real sentinels, either real or artificial, is an important method for vertebrate and vertebrate predation rates were higher (although Howe et al. (2015) set densities of artificial caterpillars to mimic natural caterpillar density. This and other aspects make it difficult to convert predation pressure on sentinel prey to “real” predation pressure in a habitat. However, the method is suitable for comparative studies (Howe et al., 2009). Notwithstanding the uncertainties and limitations, predation rates on artificial caterpillars are high, indicating a generally high predation pressure in most habitats.

Invertebrate predation was negatively related to prey size, which is consistent with the knowledge that caterpillar mortality caused by arthropods is higher in the early instars when caterpillars are smaller (Greeney et al., 2012). There is no such relationship for vertebrate predators (H3 partially supported) where such difference in size may not imply higher risk for the predator, and so does not add any protective value for the prey. However, the absence of a relationship between vertebrate predation (mostly by birds) and the size of (artificial) prey may also indicate that visually oriented predators cannot easily detect an immobile prey.

We found that contrary to expectation, invertebrate predation increases from the Equator to the poles (H4 not supported), but the apparent increase at higher latitude is greatly influenced by data from a single study in Denmark (Ferrante et al., 2014) that may or may not be typical of the situation in northern temperate forests. Further records are required, and geographical gaps have to be filled. Invertebrate and vertebrate predation rates were higher (although only significantly for vertebrates) in forests than cultivated habitats (H5 supported), suggesting that habitat complexity may be more important than habitat type for certain predators, such as birds.

The evidence here reviewed indicates that the use of sentinel prey, either real or artificial, is an important method to obtain direct quantification of predation intensity. Artificial caterpillars are as informative as real sentinels, are easy to use, and by providing additional information on predator identity, allow the partitioning of the overall predator pressure. However, in order to obtain comparable results, a standardised methodology is needed (Meyer et al., 2015). The current data on predation support the importance of top-down forces in food webs (Greeney et al., 2012).

Discussion

There are basic differences in the way research on predation using real vs. artificial prey has developed. Most of the articles using real prey come from cultivated landscapes in the U.S.A., and applications from other countries or habitats are rare. Live Lepidoptera eggs have been the most popular choice, probably for logistical reasons: they are relatively easy to produce, are often commercially available, naturally immobile, and signs of predation or parasitism on them are easy to identify. A living, mobile prey has to be constrained (e.g. Lövei & Brown, 1993), which makes the situation less “natural.” Quantitative data obtained using different prey species or stages are difficult to compare. In addition, 20% of the articles here reviewed used dead prey, which may provide a measure of scavenging rather than predation (Furlong & Zalucki, 2010), and lack of harmony in the measurement units (disappearance ≠ mortality ≠ predation) leads to further complications. Moreover, even if the number of real prey used per experiment is usually higher than for artificial ones, prey density, placement, and distribution were rarely empirically justified.

Artificial caterpillars have been more widely used, mainly in temperate and tropical forests, but increasingly in cultivated areas as well. Their most obvious advantage is the ease of manipulation, making them ideal for testing hypotheses and doing comparative studies. Few studies investigated the effect of the artificial material (Molleman et al., 2016; Sam et al., 2015b), but the reasons why a predator should attack an artificial prey have never been studied. A nonspecified amount of marks by non-predators (i.e., insects, non-predatory small rodents) found on plasticine snakes suggests that plasticine may be potentially attractive regardless of its shape (Buasso et al., 2006; Pennig et al., 2007), although this does not seem so for ants (Tvarkidova & Novotny, 2012).

We found that predation rate on real prey is higher than on artificial caterpillars (H1 supported), suggesting a possible tendency of dummy prey to underestimate predation intensity. Until specific investigations about the relationship between predation rates on real versus artificial prey are available, we can only speculate on the relative importance of each factor in such results. Vertebrate and invertebrate predation rates on artificial caterpillars were not significantly different (our H2 not supported), so possibly the similarity of an artificial caterpillar with a real prey is already sufficient to confound a vertebrate predator.

The role of naturally occurring prey on the attack rates of sentinel prey is also a potential confounding factor (Luck et al., 1988). If there are relatively low densities of naturally occurring prey, attack rates on sentinel prey may be inflated, because they are the only “prey” available. Authors rarely mentioned this in the papers reviewed, although Howe et al. (2015) set densities of artificial caterpillars to mimic natural caterpillar density. This and other aspects make it difficult to convert predation pressure on sentinel prey to “real” predation pressure in a habitat.
Conclusions

The use of artificial caterpillars is a simple, widely applicable field method that seems to be increasingly used worldwide. This method can provide valuable comparative data on predation activity. However, current practice does not allow realising its full potential, as there are too many inconsistencies in its reported use. We believe that following the recommendations below may result in better comparability between studies:

(1) Run a pilot experiment in order to find out the optimal number of sentinel prey per treatment, interprey distance and inspection time (predation rates that are too high or too low may be difficult to evaluate).

(2) When exposing the artificial sentinel prey at ground level, we recommend gluing the caterpillar onto small pieces of bamboo or reed, which allows handling the caterpillars without directly touching them. In our own experience (M. Ferrante, G. Lövei, unpublished), the superglue in gel form is easier to use, because this formulation allows a better control of the amount applied and less material is needed to fix the caterpillar to a surface. Other preparations of superglue have a tendency to react to the surface of the leaf or the caterpillar itself, and “run” – that is, quickly spread, covering a surface larger than necessary.

(3) Provide detailed information on the experimental design, including the features and placement of the artificial prey.

(4) Run independent experiments. Inspect only once, and replace all prey after a set time of inspection, irrespective of being predated or not. Reusing untouched prey, and replacing only those that are predated makes prey exposure sessions non-independent, with a loss of statistical power.

(5) Use an exposure time in multiples of 24 h.

(6) Report all the marks found (including suspected carrion feeding by non-predatory snails or slugs) with their relative “predation” rate.

(7) When presenting predation rates, always indicate sample size. A table with the number of caterpillars “attacked,” “missing,” and not attacked is of additional value. State clearly if missing caterpillars are considered lost or predated.

(8) Combining the method with various exclusion techniques can add a more sophisticated understanding of the importance of various natural enemy groups.

There remain several technical questions to be explored, including the attractiveness of the artificial material, how predators respond to real vs. artificial stimuli, as well as statistical considerations, especially the optimal distance to guarantee data independence per predatory guild. Nonetheless, the use of sentinel prey has great promise to improve our understanding of the level of predation pressure in various settings.

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Disclosure

The authors disclose no conflicts of interest of any kind associated with this manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

**Table S1.** Published data on overall predation rates on artificial caterpillars over 24 h and major predators identified, by habitat and prey position. Data are organised by habitat type, then by decreasing predation rates.

**Table S2.** Bird predation rates on artificial caterpillars over 24 h by habitat, geographical region, and prey position. Data are organised by habitat types, then by decreasing predation rates.

**References cited in the Supplementary Tables.**
Table S1. Published data on overall predation rates on artificial caterpillars over 24 h and major predators identified, by habitat and prey position. Data are organised by habitat type, then by decreasing predation rates.

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<th>Habitat type</th>
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<th>Predators identified</th>
<th>Predation partitioned</th>
<th>Prey position</th>
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<td></td>
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<td>x</td>
</tr>
<tr>
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<td>C America</td>
<td>11.0</td>
<td>x</td>
<td></td>
<td>x</td>
<td>x</td>
</tr>
<tr>
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<td></td>
<td>x</td>
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</tr>
<tr>
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<td>10.1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>s</td>
</tr>
<tr>
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<td>Tropical, elevation gradient</td>
<td>Australasia</td>
<td>10.0</td>
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<td>a, w</td>
<td>x</td>
<td>x</td>
</tr>
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<td>SE Asia</td>
<td>9.2&lt;sup&gt;1&lt;/sup&gt;</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>s</td>
</tr>
<tr>
<td>Forest</td>
<td>Tropical, forest gap</td>
<td>C America</td>
<td>9.0</td>
<td>x</td>
<td></td>
<td>x</td>
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<tr>
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<td>x</td>
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<td>Tropical, understory</td>
<td>C America</td>
<td>6.0</td>
<td>x</td>
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<td>x</td>
<td>x</td>
</tr>
<tr>
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<td>Australasia</td>
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<td>x</td>
<td>x</td>
<td>x</td>
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<td>x</td>
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<td>x</td>
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</tr>
<tr>
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<td>Tropical, 1ha fragment</td>
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<td>4.2</td>
<td>a, w</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
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<td>3.2</td>
<td>x</td>
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<td>S America</td>
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<td>x</td>
<td></td>
<td>x</td>
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<td>Cerrado</td>
<td>S America</td>
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<td>1.6</td>
<td>x</td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>
* Excluding Hymenoptera **: a: ants; r: reptiles; s: spiders; w: wasps. "Back-calculated from an original exposure time >24 h. Nc: not comparable. §predator identity was not indicated.
Table S2. Bird predation rates on artificial caterpillars over 24 h by habitat, geographical region, and prey position. Data are organised by habitat types, then by decreasing predation rates.

<table>
<thead>
<tr>
<th>Habitat type</th>
<th>Habitat detail</th>
<th>Continent</th>
<th>Country</th>
<th>Predation, % day⁻¹</th>
<th>Prey position</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Cultivated</td>
<td>Cacao plantation</td>
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<td>Indonesia</td>
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<td>Tropical, tree-poor landscape</td>
<td>Africa</td>
<td>Ethiopia</td>
<td>1.46</td>
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<td>Lemessa et al. 2015</td>
</tr>
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</tr>
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<td>Spain</td>
<td>Nc*</td>
<td>Ground</td>
<td>Church et al.1997</td>
</tr>
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<td>Temperate</td>
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<td>Canada</td>
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<td>Hossie et al., 2015</td>
</tr>
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<td>Temperate</td>
<td>N America</td>
<td>Canada</td>
<td>18.1</td>
<td>Above ground</td>
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<td>González-Gómez et al. 2006</td>
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<td>12.0</td>
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<td>Lluch et al. 2009</td>
</tr>
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<td>Chile</td>
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<td>Above ground</td>
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<td>Chile</td>
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<td>Chile</td>
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<td>González-Gómez et al. 2006</td>
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<tr>
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</tr>
<tr>
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</table>

*Nc: not comparable.  * Back-calculated from an original exposure time >24 h. §7 h exposure time.
References cited in the Supplementary Tables


Sam, K., Koane, B. and Novotny, V. (2015a) Herbivore damage increases avian and ant predation of caterpillars on trees along a complete elevational forest gradient in Papua New Guinea. Ecography, 38, 293-300.


Article II

Ferrante M, Lo Cacciato A, Lövei GL 2014
Quantifying predation pressure along an urbanisation gradient in Denmark
using artificial caterpillars
Quantifying predation pressure along an urbanisation gradient in Denmark using artificial caterpillars

MARCO FERRANTE1, ALESSANDRO LO CACCIATO2 and GABOR L. LÖVEI1

1Aarhus University, Department of Agroecology, Flakkebjerg Research Centre, Forgøsvej 1, DK-4200 Slagelse, Denmark; e-mails: marco.ferrante@agrsci.dk; gabor.lovei@agrsci.dk

2University of Palermo, Faculty of Science, Via Archirafi 28, 90123 Palermo, Italy; e-mail: locacciato-alessandro@live.it

Key words. Carabidae, Formicidae, fragmentation, natural enemies, ecosystem services, predation, urbanisation, sentinel prey

Abstract. Urbanisation results in a marked modification of habitats and influences several ecological processes, some of which give rise to beneficial ecological services. Natural pest control, the effect of predators on prey is one of such services. We quantified changes in the incidence of predation with increasing levels of urbanisation using artificial caterpillars made of green plasticine. Potential predators can be identified by the “attack marks” they leave on these artificial caterpillars. We conducted this study from May to October 2010 around the city of Sorø (Zealand, Denmark), in forests along an urbanisation gradient (rural-suburban-urban). Artificial caterpillars were placed on the ground in order to obtain an estimate of the incidence of predation at ground level. Half (50%) of the 1398 caterpillars were “attacked” and 28.8% of the bites were those of chewing insects. We attributed the majority of these to carabids, the most common group of ground-active arthropods. Chewing insects exerted the greatest predation pressure in the original forest (52.1%), with lower values recorded in the suburban (10.1%) and urban (16.4%) forest fragments. Ants were responsible for only 4.7% of the attacks in forest, 11.3% in suburban and 16.4% in urban forest fragments. Mammals exerted the highest predation pressure in suburban habitats (22.2% vs. 4.9% in forest, and 8.1% in urban forest fragments).

INTRODUCTION

Ecological services

Biodiversity provides several benefits, termed ecological or ecosystem services, which are essential for human survival (Millennium Ecosystem Assessment, 2005). The continued increase in the urban population is accompanied by marked modifications of natural areas resulting in a reduction in biodiversity, which usually negatively affects the services provided (Carpenter et al., 2009). Urbanisation is a major cause of pollution, habitat loss, extinction and impoverished biodiversity through homogenization and invasions (McKinney, 2002). However, even if the consequences of these effects are known, the quantification of ecological services is difficult and changes in the numbers of useful organisms are often used as a proxy for the level of services they provide (Isbell et al., 2011). The relationship, however, is not necessarily straightforward, with additional, controversial aspects linked to sampling, rarity, intra-guild relationships and the selection of reliable indicators (Hilty & Merenlender, 2000). Considering the environmental and economic importance of the well-being of ecosystem services for humans, it is important to identify and accurately measure ecosystem services (Layke et al., 2012; Liss et al., 2013).

Predation

Predation by natural enemies is one of the ecological services (de Groot et al., 2002), and changes in the composition and density of natural enemy groups (usually restricted to one or a few taxonomic categories) in response to urbanisation are numerous (Magura et al., 2010). However, assessing changes in the ecological function of pest control by natural enemies is neither common nor easy. Attacks by predators on invertebrate prey are usually cryptic and rarely leave any evidence, making its quantification difficult. Visual observation may be complicated, and even when possible, the presence of the observer may affect the activity of the predators. When other methods are used, such as examination of gut contents, or radioactive labelling of the prey, distinguishing between “real” predation and scavenging or secondary predation is difficult or impossible; the spread of the radioactive label may also be related to non-predatory events (Kid & Jervis, 2007). One way of measuring predation intensity is to use sentinel prey (O’Neal et al., 2005), which relies on placing prey items in a habitat, and infers predation pressure from their rate of disappearance. Suitable prey are the immobile stages of insects such as eggs (Wilson et al., 2004) or pupae (Rowe et al., 2013), or immobilised insects (e.g. aphids glued on a card, Östman et al., 2001). The disadvantage of this method is that the prey usually disappears and the identity of the predator is rarely discernible. Dummy prey can also be used, which is not removed and the marks of the attempted predation can be used to identify the predator (Howe et al., 2009). While it is not usually possible to quantify predation intensity in this way (Brodie, 1993), it is useful for comparing predation pressure in various habitats (Howe et al., 2009). Studies often indicate a high predation pressure, especially in tropical habitats (Loiselle & Farji-Brener, 2002; Posa et al., 2007; Howe et al., 2009), but there are few records from the temperate regions; published studies using dum-
my prey usually focus on predation by birds (e.g. Mäntylä et al., 2008).

Aims

Urbanisation affects the diversity and density of various groups of predators, including birds (Chace & Walsh, 2006), ground beetles (Magura et al., 2010) and spiders (Alaruikka et al., 2002; Horvath et al., 2012). However, it can only be speculated what the consequences of these structural changes on ecological functioning might be. In order to better understand the consequences of increasing urbanisation on the natural incidence of predation by ground active predators, we used the dummy caterpillar method (Howe et al., 2009) along an urbanisation gradient in Denmark. As natural habitats may offer better conditions (i.e. favourable temperature regimes, natural refuges) for predators we tested the hypothesis that the incidence of predation will diminish along a gradient of increasing urbanisation, with the highest rates recorded in forest, and lowest in patches of forest in urban parks, which corresponds to Gray’s increasing disturbance hypothesis (Gray, 1989).

MATERIAL AND METHODS

Study area

Our study area was in and around the city of Søro (55°26’N; 11°34´E; UTM: PG64), 80 km south-west of Copenhagen (Zealand, Denmark), along an urbanisation gradient selected according to the Globenet protocol (Elek & Lövei, 2005). The urbanisation gradient included rural, suburban and urban areas, each of which originated from a primary forest of beech (Fagus sylvatica) and was characterized by an increasing built-up area (0%-20%-40%) but no difference in soil characteristics (Howe & Engaard, 2006). The rural area (160 ha) was ca. 3 km west of the town centre, bordered by Lake Søro and almost completely dominated by beech, that persisted, although gradually decreasing in abundance in the other habitats. Small patches of pine (Pinus sylvestris) and other deciduous trees occurred near the forest roads. The seasonal understory of herbaceous plants consisted of Anemone silvestris, A. ranunculoides and Leucojum vernum. The suburban area (30 ha) was originally a wet forest area, now under forestry management, and close to an old cemetery. The urban area (25 ha), located 4 km from the suburban area, was an isolated park adjacent to the lake visited by many people during the year. Here the incidence of beech was lower; other trees, mostly yew (Taxus baccata) and silver lime (Tilia argentea) were more common. In each stage of the gradient, we selected four sites, each at ≥ 50 m from the next, which were further subdivided into two patches, 20 m apart. A prey patch had five individual caterpillars placed 5 m apart, which amounts to a total of 115 caterpillars per sampling occasion (the only exception was one site in the urban forest fragment, which had only one prey patch instead of two) and 1426 caterpillars during the course of this experiment. This was repeated thirteen times at approximately fortnightly intervals from the end of April until the end of October 2010.

The dummy caterpillar method

The artificial caterpillars were made of light green plasticine (Smeedi plus, V. nr. 776609, Denmark), using a modified garlic press, which produced a cylindrical shape (Howe et al., 2009), but 15 mm long and 3 mm thick as used by Tvardikova & Novotny (2012). Green colour was chosen because predators react to aposematic warning colouration or defensive signals, such as eyespots (Hossie & Sherratt, 2012). The 115 baits per sampling occasion were fixed to a suitable substrate lying on the ground (i.e. a dead leaf or dry branch) using super glue, exposed for 24 h, after which they were examined in the field using a handheld magnifying glass (12 ×). Chewing insects left characteristic mandible marks, ants numerous fine mandible marks, and small mammals their tooth marks (Fig. 1). Birds left characteristic beak marks (not illustrated). These marks were usually recognisable in the field, but in case of doubt, additional inspection was made in the laboratory using a stereomicroscope (Leica MS5, 0.63–4 × magnification).

Data analysis

The study period was divided into three seasons (spring, summer and autumn) following the “quartile method” by Fazekas et al. (1997). The number of caterpillars collected in each season was comparable (491, 456 and 451, respectively). When a caterpillar was bitten by more than one type of predator, it was included as two independent predation events. Multiple marks by the same type of predator were categorised as a single predation event. A few (n = 28, 2.0%) caterpillars were lost; we were unable to identify the predator in 1.9% of the attacks. Due to their low values, data on predation by birds and unknown predators (n = 30) were not analysed as a separate category, resulting in the 1368 caterpillars included in the analysis. We tested the probability of survival along the urbanisation gradient using the site as a random component in a generalized linear mixed model, calculated using the package “lme4” in R version 3.0.2 (R Core Team, 2013). As the random component did not affect the model, we reduced and adapted it to a logistic regression with “binomial” error distribution (link = logit). Subsequently we checked if outliers or collinearity affected the model using the package “car”. Graphs were made using the “lattice” package (Sarkar, 2008).

RESULTS

General results

Of the 1398 artificial caterpillars, 50% were attacked by predators. Chewing insects other than ants were responsible for 26.8% of the attacks on the artificial caterpillars. The other main predators were small mammals (with an
overall “predation rate” of 12.1%), ants (10.5%), and birds (0.43%).

**Predation along the urbanisation gradient**

There were different incidences in the predation of the artificial caterpillars by the different predators in the different stages of urbanisation (Fig. 2). In the forest, the caterpillars were mostly attacked by chewing insects (52.1%); ants (4.7%) and small mammals (4.9%) had a low rate of attack. In the fragments of forest in suburban areas the most common marks found belonged to small mammals (22.2%), followed by ants (11.3%), and chewing insects (10.1%). In the patches of forest in urban parks, marks inflicted by ants (16.4%) and chewing insects (16.4%) were more common than those made by small mammals (8.1%). The seasonal incidence of predation by each of the predators in these different habitats differed (Fig. 3). The incidence of predation by chewing insects in the forest habitats increased from spring to autumn. That of small mammals in the suburban area was highest in summer, followed by a decline during autumn, and that of ants in the urban area highest in spring, after which it gradually declined. The level of urbanisation affected the probability of being attacked. Caterpillars were significantly more likely to be attacked in the forest than in either of the two other stages of urbanisation (odds ratio = 2.19, 95% CI = 1.92–2.46 and odds ratio = 1.93, 95% CI = 1.68–2.18, respectively, $p < 0.001$ for both). There was no significant difference in predation pressure between the suburban vs. urban areas.

The stage of urbanisation and season had a significant effect on caterpillar “survival” (Table 1), with higher values recorded in urban and suburban areas than in forest (value = 0.801, $p = 0.000$ and value = 0.670, $p = 0.000$, respectively).

**Predation during the season**

When the stage of urbanisation was not taken into consideration, the incidence of predation by each of the main types of predator varied little from spring to autumn (Fig. 4). Marks made by ants and mammals were usually more frequently recorded during spring and summer than in autumn, while for chewing insects, the frequency was higher.

**Table 1.** The effect of the level of urbanisation on the incidence of “predation” on artificial caterpillars at Soro, Denmark, during spring-autumn of 2010, analysed using a generalized linear model. Incidence of predation in the forest and during autumn served as bases for comparison.

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>S.E.</th>
<th>Z value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.315</td>
<td>0.121</td>
<td>-2.596</td>
<td>0.009</td>
</tr>
<tr>
<td>Suburban</td>
<td>0.670</td>
<td>0.131</td>
<td>5.104</td>
<td>0.000</td>
</tr>
<tr>
<td>Urban</td>
<td>0.801</td>
<td>0.137</td>
<td>5.848</td>
<td>0.000</td>
</tr>
<tr>
<td>Spring</td>
<td>0.225</td>
<td>0.134</td>
<td>1.682</td>
<td>0.093</td>
</tr>
<tr>
<td>Summer</td>
<td>-0.481</td>
<td>0.136</td>
<td>-3.536</td>
<td>0.000</td>
</tr>
</tbody>
</table>
in summer and autumn than spring (Table 2). However, when the stage of urbanisation was considered, the incidence of predation by the different predators during the year differed (Fig. 3). Survival was significantly affected by season, being lowest in summer (value = –0.481, p = 0.000) and slightly higher in spring (value = 0.225, p = 0.093) than in autumn.

### DISCUSSION

Although there are records in the literature of the incidence of predation at ground level in a temperate region (Sipos et al., 2012), there is no information on the incidence of predation by different guilds of predators. Most of the studies carried out in the northern hemisphere using dummy prey have focused on predation by birds, with some studying defensive mechanisms such as countershading (Speed et al., 2005; Rowland et al., 2007). In our study we did not focus on any particular predator, but exploited the characteristic signs left by different predators in the malleable surface of the artificial caterpillars. The most common marks were those made by ground-active insects, ants and small mammals. The highest incidence of predation was by ground-active insects, as is recorded in other studies using artificial caterpillars (Faveri et al., 2008; Howe et al., 2009; Tvardikova & Novotny, 2012). We believe that most of the chewing insect bites were made by ground beetles (Carabidae), the most common predators in the area (Elek & Lövei, 2007). There were remarkably few attacks by birds, which are commonly recorded in tropical areas (Loiselle & Farji-Brener, 2002; Howe et al., 2009). However, these studies, although using artificial caterpillars, were carried out in different habitats using different arrangements (artificial caterpillars were fixed to leaves and not on the ground), and therefore differences in the incidence of predation may reflect different hunting strategies (Martin, 1987). Nevertheless, in our study, risk of predation was generally high, and this may have influenced habitat selection by potential prey. It is unknown whether potential prey can assess this risk and whether it influences their behaviour. We expected that due to the many different predators in forest areas, the highest incidence of predation would be recorded there, but this prediction was only partially supported. We found differences in the incidence of predation along the urbanisation gradient. In particular, the incidence of predation by ants and mammals was lowest in forest, despite the lower anthropogenic disturbance there, indicating that some predators may be favoured by human activities. However, like the trend in diversity and density of different taxonomical groups along urbanisation gradients (Magura et al., 2008), the effects of human disturbance on the incidence of predation may be related to the feeding or habitat preference of the guilds of predators studied. We recorded considerable differences in the incidence of predation when both season and stage of urbanisation were considered. This may indicate that abiotic factors differently influence the ecological services recorded in the different stages of urbanisation. Judged by the incidence of predation, suburban areas were favoured by small mammals in all the seasons studied. The same was true for chewing insects in forest and ants in urban areas, with the exception of spring and autumn, respectively. While the method is easy to use and useful in several situations (Howe et al., 2009), further refinements are needed. It is important to determine the optimal placement of the sentinel prey, and enlarge the catalogue of the different marks to identify a larger number of potential predators. For measuring ecological services in habitats subject to anthropogenic effects, it is important to control and quantify the conditions and intensity of these processes when assessing the effects of humans on non-human city dwellers, and develop countermeasures to preserve the irreplaceable services provided by biodiversity.

### ACKNOWLEDGEMENTS

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### Table 2. The number of artificial caterpillars attacked along the urbanisation gradient in three seasons by each category of predator: “ants”, “ground-active insects” and “small mammals”. Note that if an artificial caterpillar was attacked by more than one type of predator, it was counted in both categories.

<table>
<thead>
<tr>
<th>Habitat/season</th>
<th>The number of artificial caterpillars attacked by</th>
<th>No. of “unhurt” artificial caterpillars</th>
<th>Total number of artificial caterpillars exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ants</td>
<td>Ground-active insects</td>
<td>Small mammals</td>
</tr>
<tr>
<td>Forest</td>
<td>23</td>
<td>256</td>
<td>24</td>
</tr>
<tr>
<td>Spring</td>
<td>11</td>
<td>51</td>
<td>7</td>
</tr>
<tr>
<td>Summer</td>
<td>8</td>
<td>89</td>
<td>5</td>
</tr>
<tr>
<td>Autumn</td>
<td>4</td>
<td>116</td>
<td>12</td>
</tr>
<tr>
<td><strong>Suburban</strong></td>
<td><strong>55</strong></td>
<td><strong>49</strong></td>
<td><strong>108</strong></td>
</tr>
<tr>
<td>Spring</td>
<td>25</td>
<td>14</td>
<td>38</td>
</tr>
<tr>
<td>Summer</td>
<td>24</td>
<td>19</td>
<td>48</td>
</tr>
<tr>
<td>Autumn</td>
<td>6</td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td><strong>Urban</strong></td>
<td><strong>69</strong></td>
<td><strong>69</strong></td>
<td><strong>36</strong></td>
</tr>
<tr>
<td>Spring</td>
<td>32</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Summer</td>
<td>33</td>
<td>33</td>
<td>14</td>
</tr>
<tr>
<td>Autumn</td>
<td>4</td>
<td>26</td>
<td>8</td>
</tr>
<tr>
<td><strong>Grand Total</strong></td>
<td><strong>147</strong></td>
<td><strong>374</strong></td>
<td><strong>168</strong></td>
</tr>
</tbody>
</table>
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**ORIGINAL CONTRIBUTION**

**Manipulating field margins to increase predation intensity in fields of winter wheat (Triticum aestivum)**

A. Mansion-Vaquíé¹, M. Ferrante¹, S. M. Cook², J. K. Pell², a & G. L. Lövei¹

¹ Department of Agroecology, Flakkebjerg Research Centre, Aarhus University, Slagelse, Denmark
² AgroEcology Department, Rothamsted Research, Harpenden, Herts, UK

**Keywords**
arthropod predation, artificial caterpillars, cereal aphids, conservation biological control, flower strips, sentinel prey

**Correspondence**
Gabor L. Lövei (corresponding author), Department of Agroecology, Flakkebjerg Research Centre, Aarhus University, Forsøgsvej 1, DK-4200 Slagelse, Denmark. E-mail: gabor.lovei@agro.au.dk

aCurrent Address: J.K. Pell, Consulting, Luton, Beds., UK

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**Abstract**
The effectiveness of natural enemies to control pests can be enhanced through habitat manipulation. However, due to the differences in their ecology, generalist and specialist species may respond differently to the same manipulation. Moreover, interactions among natural enemies (i.e. cannibalism, intraguild predation, hyperparasitism) may complicate the assumption that a higher density of natural enemies would increase the level of biological control. We investigated the natural enemy guild composition and the predation rate along flower vs. grass margins at the edge of winter wheat (Triticum aestivum) fields in Denmark. Natural enemies were sampled by pitfall trapping and by suction sampling; predation intensity was measured using two different sentinel prey methods: artificial caterpillars made of plasticine, and sentinel aphid colonies. Specialist and generalist species responded differently to the two margin types: specialists (mostly parasitic wasps) were attracted by the flower margins, while generalists (ground beetles, rove beetles and spiders) were more active in grass margins. The number of artificial caterpillars attacked was significantly greater in grass margins (mean = 48.9%, SD = 24.3) than in flower margins (mean = 30.7%, SD = 17.4). We found a significant positive relationship between the number of artificial caterpillars attacked by chewing insects, and activity density for large (≥15 mm) ground beetles. Predation of sentinel aphids in wheat fields did not vary significantly in relation to margin type. Our results suggest that flowering margins may be beneficial for canopy-active specialist natural enemies, but grassy margins are more useful for ground-active generalist predators.

**Introduction**
Conservation biological control (CBC) intends to restore and sustain the activity of natural enemies that control crop pests (Eilenberg et al. 2001). Natural enemy communities can significantly decrease pest abundance (Symondson et al. 2002), especially in wheat monocultures that are mainly infested by indigenous herbivorous pests (Pedigo and Buntin 1993). Such natural enemy communities include generalist and specialist species that differ in their range of prey/hosts (Welch et al. 2012). Despite their different performance as biological control agents, both have important roles in controlling pest populations (Southwood and Comins 1976; Symondson et al. 2002; Welch et al. 2012). In winter wheat fields, parasitic wasps (Hymenoptera: Braconidae and Ichneumonidae) are effective natural enemies of aphids (Schmidt et al. 2003), and in this article, we label them ‘specialists’, while spiders (Araneae), carabids (Carabidae) and rove beetles (Staphylinidae) were considered generalist predators because they regularly prey on other organisms as well as aphids (Symondson et al. 2002; Welch et al. 2012).
Conservation biological control strategies often involve the manipulation of non-crop habitats surrounding fields to protect, enhance or recreate the conditions necessary for the persistence and activity of natural enemies (Barbosa 1998; Landis et al. 2000; Eilenberg et al. 2001). Field margins are areas of uncropped land at the edges of crops adjacent to their boundaries, and are sometimes specifically managed to make them flower-rich (Landis et al. 2005). They favour the establishment and survival of autochthonous beneficial arthropods by providing shelter with appropriate microclimatic conditions (Landis et al. 2000; Griffiths et al. 2008), or additional resources such as alternative prey, pollen and nectar (Marshall and Moonen 2002; Landis et al. 2005; Griffiths et al. 2008). The presence of flowers adjacent to crops can promote a higher level of pest control compared to grass (Blaauw and Isaacs 2012; Balzan and Moonen 2014). This is usually attributed to the presence of floral resources (pollen, nectar and sometimes extra-floral nectar) that may be necessary during particular life stages of natural enemies such as hoverflies, lacewings, ladybirds and some spiders (Wäckers et al. 2008; Lu et al. 2014).

As a consequence of supporting more natural enemies (Dennis and Fry 1992), manipulated field margins are expected to improve biological control of pests in the adjacent fields. However, studies examining the effect of habitat manipulation on the level of biological control often lack a quantitative assessment of predation intensity in the field (Jervis and Kidd 1996; Howe et al. 2009), limiting the investigation to the natural enemy community (e.g. predator: prey ratio) rather than quantifying their positive function with respect to pest control (Pedigo and Buntin 1993; Griffiths et al. 2008). Interactions among natural enemies (i.e. cannibalism, intraguild predation, hyperparasitism) may complicate the basic assumption that more predators will result in a higher level of biological control (Letourneau et al. 2009). Sentinel prey methods are promising approaches to measure the in-field predation rate of natural enemies. To date, only a few studies have investigated the impact of habitat manipulation or naturally occurring non-crop habitats around the crop fields on the natural regulatory activity of beneficial arthropods. These often use sentinel prey, such as egg clutches (Thomson and Hoffmann 2010; Balmer et al. 2013), aphids (Griffiths et al. 2008; Holland et al. 2008; Rusch et al. 2013) or artificial caterpillars (Howe et al. 2015) to estimate efficacy.

The aim of this study was to characterize assemblages of natural enemies to obtain quantitative estimates of their effect in cultivated fields adjacent to different field margin types (flower or grass). We hypothesized that (H1) a higher abundance of alternative food subsidies in sown flower margins would support a higher abundance of natural enemies in those margins than in grass margins. We further hypothesized that (H2) immigration of natural enemies from the flower-rich margins would be greater than that from grass margins resulting in larger populations of natural enemies in the crop in the former compared with the latter. Finally, we tested the hypothesis that (H3) predation pressure would be greater in the areas of the crop close to flower margins than in areas close to grass margins.

Materials and methods

Study site and field experimental design
The study was carried out at the Flakkebjerg Research Station Experimental Farm (Aarhus University), in the Vestsjælland Region of Denmark. In the autumn of 2013, a seed mixture composed of an equal proportion of two cruciferous plants (Brassica rapa var. rapa and Raphanus sativus var. oleiformis) was sown along a 50 m long, 2.5 m wide strip at the edges of five winter wheat fields (mean = 6.7 ha, SD = 3.3 ha) (Figure S1). We chose these species as previous studies showed a differential in flowering time (B. rapa flowering early, and R. sativus flowering relatively later, S. Cook, unpublished data), and they attract a range of natural enemies, including aphid parasitoids (Cook et al. 2013), while they support no herbivorous pests of wheat. Grass margins were selected on the opposite side of the same fields, away from other flowering areas, and were regularly cut, to ensure no flower resources were present. During the 2014 season, the flower margins, grass margins and the crop strips were sampled to compare the effect of the margin type on predation rates/intensity and on the composition of natural enemy communities. In one field, only the grass margin was sampled due to the failure of the flower margin to establish.

Arthropod sampling methods to determine natural enemy community composition
The composition of the arthropod natural enemy community was evaluated using two complementary sampling methods. Epigeal predators were sampled using four pairs of pitfall traps (500 ml volume and 10 cm diameter, filled with 100 ml ethylene glycol 70%) per field; two pairs 5 m apart in the crop strip...
sampled adjacent to the grass margin, and two pairs 5 m apart in the crop sampled adjacent to the flower margin. The trap pairs were placed 12.5 m from the field edge, at the tips of a plastic fence arranged in a zig-zag pattern (one side 30 cm long, 20 cm tall, dug into the soil 10 cm deep; one part of the fence was open in a V-shape towards the crop centre, and the other towards the field margin, see Figure S1b). Barrier pitfall traps in general are more effective sampling devices than single pitfalls (Hansen and New 2005), but our arrangement had an additional advantage: one of the pitfalls caught mostly arthropods moving into the crop from the margin (immigrating), while the other mainly caught arthropods presumably leaving the crop. Each of the five fields therefore had eight traps in total except for the field in which the flower margin failed (36 traps per sample in total). To reduce the bycatch, each trap was covered by a square of galvanized iron (10 × 10 cm), supported by pegs. The traps were open for three periods of 7 days each, separated by 1 week (21–28 May, 3–10 June, 18–25 June and 2–9 July 2014). A total of 144 samples were collected using this method.

Arthropods active in the crop and margin vegetation were collected using a vacuum suction sampler constructed from a modified portable leaf blower (Husqvarna® 125BVx). The vacuum tube was 85 cm long and 12.5 cm diameter. Suction samples were collected by walking along a 15 m transect within each margin and the crop strips (Figure S1b). Every 3 m, the vacuum tube was placed onto the soil surface and kept there for 10 s before continuing the walk. After finishing one transect, the collected material (i.e. from five positions) was transferred into a large plastic bag, placed in a cooler box and transferred to the laboratory. Samples were stored at −20°C before sorting. Suction sampling was carried out fortnightly on 3 June, 16 June and 1 July 2014, resulting in a total of 54 samples (crop area associated with the failed margin was not sampled). All collected arthropods were identified to order or, in the case of beetles (Coleoptera), to family using the identification keys of Choate (1999) and Unwin (1981). We considered predatory beetles, spiders and harvestmen generalist natural enemies of aphids, while parasitoids, syrphid larvae, lacewings and coccinellids were categorized as aphid specialists.

Quantifying predation intensity

Predation pressure was quantified using two kinds of sentinel prey: populations of the grain aphid (Sitobion avenae) acting as live sentinel prey and artificial caterpillars made of green plasticine (Howe et al. 2009; Ferrante et al. 2014). Live aphid prey patches were established in three different types of exclusion cage per margin: open, partially closed and totally closed. They were placed in a random sequence in the crop strips, 5 m from each other. Cages were cylindrical (31.5 cm diameter, 50 cm height) with a solid plastic frame and a mesh cover. The open cage consisted of a completely uncovered frame, with no mesh, allowing access to the sentinel prey by all natural enemies. The partially closed cage was covered by a plastic mesh 2 × 2 cm size, left uncovered at the bottom, which excluded large potential natural enemies such as rodents or birds. The total exclusion cage was fully covered with muslin mesh (<1 mm) designed to exclude all natural enemies and was therefore used as the control. To ensure that no natural enemies could enter the total exclusion cage, two muslin mesh covers were used. The ‘top cover’ was glued to the frame of the cage, and a ‘bottom cover’ mesh was placed on the ground and brought up to overlap the top cover. We considered a set of three cages as one replicate. Each cage contained a pot with ca. 20 greenhouse-grown winter wheat plants, 10 cm tall, infested with ten grain aphids of mixed age (nymphs and adults). Aphids were transferred onto a single wheat leaf using a paintbrush (while in the greenhouse). The pots were transferred to the field and dug into the soil so that ground-active organisms had level access to the plants (except for the total exclusion cages, where the pot was put on the mesh at the base). The fate of these aphid colonies was followed during the flowering period of the flower margin, with non-destructive counting of living aphids twice each week. If a population of aphids did not establish (after being transferred to the field) in the totally or partially closed cage, a new replicate was performed; that is, the set of the three different cages was re-installed and restarted for each margin on that field. In total, 25 such replicates were run in the five experimental wheat fields from 7 June to 7 July 2014.

Artificial sentinel prey consisted of light green plasticine (Smeedi plus, V. nr. 776609, Denmark ‘caterpillars’ 15 mm long and 3 mm thick (Howe et al. 2009). This method allows the identification of up to 14 different types of predators (Low et al. 2014; Lövei and Ferrante, 2017). Each caterpillar was glued onto a small piece of reed or bamboo, to be handled without touching the plasticine. We placed 15 caterpillars on the ground along the margins and also along the crop strips (Figure S1b; i.e. a total of 60 caterpillars per field per sampling event), and these were observed after 24 h for signs of predation attempts. Artificial
caterpillar experiments were run weekly (26 May, 4 June, 12 June, 18 June, 25 June, 3 July 2014), covering the period when the pitfall traps were active.

Statistical analysis

Community composition of natural enemies
We tested for differences in natural enemy abundance between flower margins and grass margins using Student’s paired t-tests. All data were normalized using a log10(x + 1) transformation. For each sampling method, taxonomic orders representing <1% of the total catch (Diptera, Neuroptera, Chilopoda, Coccinellidae and Cantharidae, see Tables S1, S2) were excluded from the analysis. The remaining natural enemies collected were divided into two guilds: generalists (Coleoptera, Araneae, Opiliones) and specialists (Hymenoptera).

Sentinel aphids
Of the total of 25 aphid cage replicates, four (two for each of the two margin types) were excluded from the analysis because the control population (aphids in the total exclusion cage) also went extinct. As the replicates were run until the population of aphids went extinct in both open and partially closed cages (or one being extinct and the other one reaching a very low number of 1 or 2 aphids), the length of the observation periods varied. Therefore, when necessary, we interpolated the number of aphids after 2 and 5 days from the start of each replicate by connecting data points with a straight line, and reading the resulting value. To determine how the provision of the biological control service was affected by the margin treatments (flower or grass margins), the degree of aphid suppression was calculated in each margin by expressing the change in aphid numbers in open and partial exclusion cages as a proportion of aphid abundance compared to numbers reached in the absence of predators (numbers in the total exclusion cages). The resulting Biocontrol Service Index (BSI, Gardiner et al. 2009) is defined as

\[ BS1 = \frac{A_c - A_o}{A_c} \]

where \( A_c \) is the number of aphids on the caged plant (total exclusion cage) after 2 or 5 days from inoculation, and \( A_o \) is the number of aphids on the open plant (open or partial exclusion cage) on day 2 or day 5. Values of BSI can range from 0 to 1, with values increasing as the level of aphid predation increases. As suggested by Gardiner et al. (2009), cases with negative BSI values indicate a lack of effective biocontrol.

To assess the influence of the nature of the margin on the biological control service, BSI values were compared between margin types and among cages within the same margin of the same field, using the Wilcoxon signed-ranks test (W). The absence of aphid predation was compared using the odds ratio (Rita and Komonen 2008). The lifetime of the aphid colonies, represented by the number of days before the colony went extinct, was analysed using the Wilcoxon–Mann–Whitney U-test.

Artificial caterpillars
Predation rate was analysed as a response in a linear mixed model including three fixed factors, type of margin (grass vs. flower), position in the field (crop vs. margin), phenological period (before vs. after flowering of the margin (flowering: 26 May, 4 and 12 June vs. after flowering: 18, 25 June, 3 July)), and all their interactions; field was used as a random factor. As the random factor explained <1% variance, it was excluded and the model was simplified to a linear model including the aforementioned factors and their interactions. The best model was decided using backward selection and by comparing Akaike Information Criteria and included the three fixed factors and the interaction between margin and phenological period, and margin and position. Model residuals were checked to verify that the parametric assumptions of normality and homoscedasticity were satisfied. Model validation was carried out graphically. Missing caterpillars were considered lost and were excluded from the analyses. Finally, we tested the relationship between predation rate on artificial caterpillars by chewing insects and the activity density of carabids \( \geq 15 \text{ mm} \) in length, using a simple linear regression. Ground beetles \( <15 \text{ mm} \) were removed from the analyses, as it was assumed that they would not attack prey larger than themselves. Artificial caterpillars were always set up while pitfall traps were active, but as the two sampling methods had a different number of runs (six and four, respectively), we used the mean predation rate percentage in two cases. As we only had data for the grass margin in one field, this field was not considered in the analysis. Ground beetle activity density was log-transformed to meet the parametric assumptions. All statistical analyses were performed using the statistical program R, version 3.1.1 (R Core Team, 2014).

Results

Arthropod community composition
A total of 9640 (Table S1) and 1875 (Table S2) natural enemies were collected by pitfall trapping and by suction sampling, respectively. Generalist natural
enemies of aphids represented the majority of the catch from pitfall traps, with beetles (Coleoptera: 66.3%) and spiders (Araneae: 23.6%) being the most numerous, while specialist natural enemies of aphids (Hymenoptera) represented only 9.2% of the arthropods in pitfall traps. Hymenoptera represented the majority of the catch (62.5%) from vacuum suction sampling, in which the generalist natural enemies represented 37.0%, including Coleoptera (20.8%), Araneae (13.7%) and Opiliones (2.2%). Predatory Diptera collected (by both methods) were robber flies (Asilidae), and predatory Neuroptera were lacewings (Chrysopidae). Robber flies, lacewings and centipedes (Chilopoda) represented <1% of the catch.

The abundance of ground beetles and spiders collected by pitfall traps was not correlated (Pearson’s $r = -0.07$). However, spider abundance was negatively related to ground beetle abundance except on the first sampling occasion (fig. 1), although it was never statistically significant. This is because under low carabid abundance, spider abundance varied greatly. It is noticeable, though, that high carabid abundance was usually accompanied with low spider abundance, and high spider abundance was only found when carabid abundance was low (the slope of the upper envelope of fig. 1 is steeply negative).

Effect of flower vs. grass margins on the abundance of natural enemies

Overall, the flower margins supported a significantly greater abundance of specialist natural enemies (collected by vacuum suction sampling) compared to the grass margins (Student’s $t$-test, $t = 2.42$, d.f. = 11, $P = 0.03$; fig. 2). Grass margins supported a significantly greater abundance of generalist natural enemies (measured by pitfall trap catches) compared to

![Fig. 1 Relationship between ground beetle and spider abundance (no. individuals per trap) in winter wheat crops with grass and flower margins during the 2014 field season in Flakkebjerg, Denmark.](image)
the flower margins over the whole season (Student’s t-test, \( t = -4.33, \text{d.f.} = 11, P = 0.001 \)); this difference mainly driven by significantly greater numbers of generalists found in grass than flower margins in mid-June (Student’s t-test, \( t = 7.37, \text{d.f.} = 3, P = 0.005 \); fig. 2). This difference between margin types did not translate into a significant difference in the abundance of natural enemies between the crop strips of the fields for either generalists (Student’s t-test, \( t = -1.10, \text{d.f.} = 11, P = 0.30 \)) or specialists (Student’s t-test, \( t = -0.41, \text{d.f.} = 11, P = 0.69 \)). The activity density of generalist and specialist natural enemies in the pitfall traps in the crop was also not influenced by the type of margin (Student’s t-test, \( t = -0.02, \text{d.f.} = 61, P = 0.99 \) and \( t = 0.08, \text{d.f.} = 61, P = 0.94 \), respectively) except on two occasions. More generalists were found in pitfall traps in late May in the crops near flower margins compared with those near grass margins (Student’s t-test, \( t = 2.02, \text{d.f.} = 15, P = 0.06 \)), while in July, the opposite was found (Student’s t-test, \( t = -3.25, \text{d.f.} = 13, P = 0.006 \); fig. 3). Specialists caught by pitfall traps were low in abundance and did not differ according to the direction of pitfall traps (fig. 3).

Influence of margin type on the distribution of natural enemies

The data from the suction sampling indicated that in both types of margins, the abundance of specialist natural enemies was significantly greater in the margin than the crop throughout the whole season (Student’s t-test, \( t = 4.38, \text{d.f.} = 26, P = 0.0002 \); fig. 2). The abundance of the generalist predators did not significantly differ between crop and margin for either margin type (Student’s t-test, \( t = 0.24, \text{d.f.} = 26, P = 0.81 \); fig. 2). Generalists tended to be more abundant in the crop associated with flower margins (significant only in July, Student’s t-test, \( t = 3.85, \text{d.f.} = 3, P = 0.03 \)) and in the grass margins after mid-June (fig. 2). Directional pitfall traps showed no significant immigration or emigration of natural enemies except in late May, with significantly more individuals caught in the emigration than immigration traps near the flower margin (Student’s t-test, \( t = 3.25, \text{d.f.} = 7, P = 0.01 \); fig. 3). There was also a marginally significant sink effect of the grass margin on generalist predators at the beginning of July (Student’s t-test, \( t = 2.09, \text{d.f.} = 9, P = 0.07 \); fig. 3).
Influence of field margin composition on aphid predation intensity

The average lifetime of the aphid colonies was significantly longer in the grass margin treatment (9.9 days) compared with the flower margin treatment (5.8 days) (Wilcoxon–Mann–Whitney test, \(U = 7.5, P = 0.017\)), but there were no differences between the open vs. partial exclusion cages (flower margins: Wilcoxon–Mann–Whitney test, \(U = 5.5, P = 0.224\), grass margins: \(U = 9, P = 0.815\)). The value of the BSI significantly increased between day 2 and day 5 (Table S3, Wilcoxon signed-rank test, \(W = 129, P = 0.001\)). The type of margin did not influence the provision of the biological control service, with no difference in the BSI values when the same type of exclusion cages were compared adjacent to the grass vs. flower margins, either after 2 days (Table S3, Wilcoxon signed-rank test, \(W = 59, n = 14, P = 0.706\)), or after 5 days (Wilcoxon signed-rank test, \(W = 53, n = 14, P = 0.625\)). Open and partially closed cages showed a similar BSI after 2 days (grass margin treatment, Wilcoxon signed-rank test, \(W = 19, P = 0.093\), flower margin treatment, \(W = 16, P = 0.834\)) and after 5 days (grass margin treatment \(W = 47, P = 0.23\); flower margin treatment, \(W = 13, P = 1.0\)).

In the flower margin treatment, the ratio of cages that showed no biocontrol effect (BSI = 0) after 2 days was 17%, and after 5 days, this increased to 28%. In the grass margin treatment, the opposite was found: an absence of aphid biocontrol effect was recorded in 33% of the cages after 2 days and only in 25% after 5 days. The absence of aphid biocontrol effect was not significantly different by margin treatment (odds ratio after 2 days = 2.00, CI95% = 0.46–8.62, \(P = 0.35\) and odds ratio after 5 days = 0.90, CI95% = 0.24–3.42, \(P = 0.88\)).

Influence of field margin composition on predation on artificial caterpillars

Forty-six per cent \(n = 756/1636\) of the artificial sentinel prey were attacked after 24 h, mostly by chewing insects (88%, \(n = 665/756\) of the bites), followed by small mammals (13.2%), and birds (1.3%). Fourteen caterpillars (0.79%) were lost. Predation rate by chewing insects was higher in grass than in flower margins (48.9%, SD = 24.3, \(n = 30\) vs. 30.7%, SD = 17.4, \(n = 25\), respectively) and was also higher in the margins than within the crop (45.3%, SD = 27.3, \(n = 30\) vs. 35.9% SD = 19.3, \(n = 30\), respectively). In the flower margin treatment,
predation was similar between the crop and the margin (30.9%, SD = 18.1, n = 25 vs. 30.6%, SD = 23.2, n = 25, respectively), while in the grass margin treatment, it was higher in the margins than in the crop (57.6% SD = 31.9, n = 30 vs. 40.1% SD = 24.5, n = 30, respectively). The multiple linear regression (adj. R² = 0.33) indicated that total predation was significantly higher in grass than in flower margins (Table 1) and was also significantly higher after flowering than during flowering (Table S1). Moreover, the interaction between flowering period and margin type was significant (Table S1) as predation in the grass margin was significantly lower during than after flowering (Table S1, fig. 4). We found a significant positive relationship (t = 5.616, P < 0.01, adj. R² = 0.50) between the activity density of large (≥15 mm) ground beetles and the attack rate on artificial caterpillars (fig. 5).

**Discussion**

Field margin manipulation is one of the most promising practices to enhance biological control in cultivated crops (Landis et al. 2000). However, the influence of the various groups within the natural enemy community on pest populations in adjacent crops still requires clarification (Pfiffner and Wyss 2004). In our study, grass and brassica-containing flower margins had different impacts depending on the natural enemy groups. Our hypothesis 1 (that a higher abundance of alternative food subsidies in sown flower margins would support higher numbers of natural enemies compared with grass margins) was only partially supported, as flower margins increased the abundance of specialist but not generalist natural enemies. Apparently, the structurally complex grass edge provided sufficient attraction to ground-active generalist natural enemies, to which flowers added little attraction. For ground-active predators, ground cover structure (possibly by influencing humidity) may be more important (Frank and Reichhart 2004; Woodcock et al. 2005) than other factors, at least in the first part of the growing season (note wheat is harvested in early July, that is the middle of the northern summer, when the activity period of the natural enemies is far from its end). The increase of specialists in flower margins did not translate to an increased abundance in the crop, so our second hypothesis (that immigration of natural enemies from the flower-rich margins would result in larger populations of natural enemies compared with crops adjacent to grass margins) predation was not supported, at least under Danish conditions. In a similar study, the abundance of aerial natural enemies (including specialists such as Aphidiinae) in winter wheat fields in the UK significantly increased in the presence of flower margins, while grass margins had only a small effect (Ramsden et al. 2015). In our study, generalist arthropods were more abundant in grass than in flower margins, and grass margins may represent a source of generalists migrating towards the crop. However, at the beginning of the experiment, generalist predator abundance was higher in flower margins than in grass margins.

![Fig. 4](image-url) Probability of predation on artificial caterpillars during the 2014 cropping season in Flakkebjerg, Denmark, estimated using a linear model. Circles and bars indicate fitted values and confidence intervals, respectively.
margins, suggesting that a positive effect may exist only until alternative optimal prey becomes available. Despite studies describing an increase in arthropod species diversity and abundance (Haaland et al. 2011), studies comparing flower margins with grass margins also indicate that carabids and spiders do not show a particular preference for any kind of margin (Meek et al. 2002; Pfiffner and Wyss 2004).

The positive BSI obtained using the sentinel aphid method suggests effective control of aphids in fields with either type of margin. The positive influence of the flower margin can be seen in the fate of aphid colonies: aphid survival was shorter adjacent to flower than grass margins. This response is in line with our third hypothesis, predicting a higher level of biocontrol near flower than grass margins. However, there were more generalist predators in grass than flower margins, and artificial prey were more attacked by generalists there, indicating higher predation pressure by generalists near grassy margins. These data do not support our third hypothesis. Predation on artificial caterpillars may reflect the activity of only a part of the natural enemy community, as not all will attack such potential prey (the size of the sentinel prey may discourage small predators (Lövei and Ferrante 2017), and parasitoids rarely attack such artificial prey (Howe et al. 2009)). Nonetheless, our results confirm the applicability of the method to European agroecosystems, which to date have been used only in tropical agroecosystems (Gray and Lewis 2014; Howe et al. 2015; Maas et al. 2015). An increase in predator abundance in crops neighbouring flower strips does not necessarily translate to increased biological control (Markó et al. 2012; Cox et al. 2014). The effectiveness of flower margins as a source of parasitoids for aphid control in adjacent crops may be strongly limited by the margin-crop distance. Distances over 2 m may already show almost no effect on the abundance of parasitoids (Bianchi and Wäckers 2008) or their parasitism (Tylianakis et al. 2004) in the crop. Other groups of natural enemies, such as hoverflies, show greater dispersal activity (Lövei et al. 1998) and can be linked to increased biocontrol (Hickman and Wratten 1996).

Predation impact did not differ between open (all predators had access to live sentinel aphids) and partial exclusion cages (only invertebrate natural enemies have access), indicating that invertebrate predators were mostly responsible for aphid control in winter wheat fields. The significant relationship between the abundance of large carabids and attack rates on the artificial caterpillars indicates that the natural enemy role of ground beetles can be important. The negative relationship between carabids and spiders may have been caused by different seasonal phenologies, but the more probable explanation is intraguild predation (Lang 2003; Schmidt et al. 2003). This also points to the complexity of the natural enemy impact, underlining that abundance measures only may not provide the true picture of such impact.

**Conclusions**

Flowering margins may benefit natural enemies, but our results suggest that they influence generalist and specialist natural enemies differently. As both may be important in pest regulation, agro-environments should be managed as a mosaic of different habitats, which may include flower margins and grassy strips, to provide abiotic and biotic resources to the whole natural enemy community. To date, predation rates in agroecosystems have been measured using artificial caterpillars only in tropical areas (Lövei and Ferrante 2017). Our results confirm the applicability of the method in European agroecosystems. The partial overlap between abundance patterns in the natural enemy community and the measures of their functionality suggests that both live and artificial sentinel prey are informative and ecological studies focusing on biological control should include both. To evaluate the impact of habitat manipulation on biological control, we recommend measuring predation intensity together with traditional investigations of natural enemy densities and community composition.
Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. (a) Schematic representation of the field experiment design; the shaded box represents a grass margin; the stippled box represents a flower margin, white areas represent areas of crop sampled. (b) Spatial locations and types of sampling in crop and field margins.

Table S1. Mean activity-density (no. of individuals trap⁻¹ day⁻¹ ± SE) collected by directional pitfall traps, representing emigration (out) and immigration (in) in wheat fields with adjacent flower (n = 8) and grass margins (n = 10) on the four sampling events during the spring-summer season, 2013 in Flakkebjerg, Denmark.

Table S2. Mean number of individual arthropods (±SE) caught by suction sampling in flower margins (n = 4), grass margins (n = 5), and in adjacent areas of
the wheat crop during the spring-summer season, 2014 in Flakkebjerg, Denmark.

**Table S3.** Descriptive characteristics of the Biocontrol Service Index (BSI) in the aphid sentinel prey cages with grass or flower margin treatments, 2 days and 5 days after the sentinel aphid colony establishment.
**Table S1:** Mean activity-density (no. of individuals trap\(^{-1}\) day\(^{-1}\) ± S.E.) collected by directional pitfall traps, representing emigration (out) and immigration (in) in wheat fields with adjacent flower (n = 8) and grass margins (n = 10) on the four sampling events during the spring-summer season, 2014 in Flakkebjerg, Denmark.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>21-28 May</th>
<th>3-10 June</th>
<th>18-25 June</th>
<th>2-9 July**</th>
<th>21-28 May</th>
<th>3-10 June</th>
<th>18-25 June</th>
<th>2-9 July**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coleoptera</td>
<td>Out</td>
<td>In</td>
<td>Out</td>
<td>In</td>
<td>Out</td>
<td>In</td>
<td>Out</td>
<td>In</td>
</tr>
<tr>
<td></td>
<td>105.0±5.5</td>
<td>91.5±4.2</td>
<td>40.3±1.5</td>
<td>40±2.2</td>
<td>24.0±1.5</td>
<td>23.9±1.8</td>
<td>13.6±1.1</td>
<td>13.9±1.2</td>
</tr>
<tr>
<td>Carabidae</td>
<td>Out</td>
<td>In</td>
<td>Out</td>
<td>In</td>
<td>Out</td>
<td>In</td>
<td>Out</td>
<td>In</td>
</tr>
<tr>
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<td>In</td>
<td>Out</td>
<td>In</td>
<td>Out</td>
<td>In</td>
<td>Out</td>
<td>In</td>
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<td>Araneae</td>
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<td>Out</td>
<td>In</td>
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<td>Out</td>
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<td>Opiliones</td>
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<td>In</td>
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<td>In</td>
</tr>
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<td>0.1±0.04</td>
<td>0.1±0.04</td>
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| Total               | 132.6±5.0 | 112.0±4.5 | 58.3±2.2   | 56.0±2.3   | 46.9±3.0  | 50.4±4.0 | 45.3±2.8   | 37.1±1.5   |

*Taxonomic orders representing less than 1% of the total.

** Due to the loss of one pitfall trap, n=7 on this date only.
Table S2: Mean number of individual arthropods (± S.E.) caught by suction sampling in flower margins (n = 4), grass margins (n = 5), and in adjacent areas of the wheat crop during the spring-summer season, 2014 in Flakkebjerg, Denmark.

<table>
<thead>
<tr>
<th>Taxon</th>
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<th></th>
<th>Grass margin</th>
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<td></td>
<td>03 June</td>
<td>16 June</td>
<td>01 July</td>
<td>03 June</td>
</tr>
<tr>
<td></td>
<td>Margin</td>
<td>Crop</td>
<td>Margin</td>
<td>Crop</td>
</tr>
<tr>
<td>Hymenoptera</td>
<td>26.8±6.4</td>
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<td>72.0±13.4</td>
<td>25.0±4.1</td>
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<td>Coleoptera</td>
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<td>0.5±0.1</td>
<td>0.3±0.1</td>
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<tr>
<td>Carabidae</td>
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<td>0</td>
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<td>Staphylinidae</td>
<td>3.0±0.5</td>
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<tr>
<td>Coccinellidae*</td>
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<td>0</td>
</tr>
<tr>
<td>Cantharidae*</td>
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<td>0.3±0.1</td>
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</tr>
<tr>
<td>Araneae</td>
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<td>Opiliones</td>
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<td>Diptera*</td>
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<tr>
<td>Total</td>
<td>32.0±6.9</td>
<td>10.3±2.0</td>
<td>74.0±13.2</td>
<td>28.8±4.2</td>
</tr>
</tbody>
</table>

*Taxonomic orders representing less than 1 % of the total.
**Table S3.** Descriptive characteristics of the Biocontrol Service Index (BSI) in the aphid sentinel prey cages with grass or flower margin treatments, 2 days and 5 days after the sentinel aphid colony establishment. Note that due to non-normal distribution, medians, quartiles and ranges are given.

<table>
<thead>
<tr>
<th>Treatment/Cage</th>
<th>Mean after 2 days</th>
<th>Mean after 5 days</th>
<th>Median after 2 days</th>
<th>Median after 5 days</th>
<th>Lower quartile after 2 days</th>
<th>Lower quartile after 5 days</th>
<th>Upper quartile after 2 days</th>
<th>Upper quartile after 5 days</th>
<th>Maximum* after 2 days</th>
<th>Maximum* after 5 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flower edge Open</td>
<td>0.47</td>
<td>0.59</td>
<td>0.60</td>
<td>0.69</td>
<td>0.26</td>
<td>0.59</td>
<td>0.67</td>
<td>0.83</td>
<td>0.80</td>
<td>0.87</td>
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<tr>
<td>Partial exclusion</td>
<td>0.50</td>
<td>0.54</td>
<td>0.53</td>
<td>0.82</td>
<td>0.20</td>
<td>0.00</td>
<td>0.80</td>
<td>0.96</td>
<td>0.86</td>
<td>1.00</td>
</tr>
<tr>
<td>Grass edge Open</td>
<td>0.46</td>
<td>0.49</td>
<td>0.54</td>
<td>0.53</td>
<td>0.13</td>
<td>0.25</td>
<td>0.71</td>
<td>0.83</td>
<td>0.86</td>
<td>0.92</td>
</tr>
<tr>
<td>Partial exclusion</td>
<td>0.23</td>
<td>0.40</td>
<td>0.13</td>
<td>0.37</td>
<td>0.00</td>
<td>0.13</td>
<td>0.48</td>
<td>0.75</td>
<td>0.69</td>
<td>0.86</td>
</tr>
</tbody>
</table>

* the minimum values were 0.00 in all treatments; 1 accessible to all predators; 2 only accessible to small (<30 mm) invertebrate predators.
Figure S1: (A) Schematic representation of the field experiment design; the shaded box represents a grass margin; the stippled box represents a flower margin, white areas represent areas of crop sampled. (B) Spatial locations and types of sampling in crop and field margins.
Article IV

Ferrante M, González E, Lövei GL
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Predators do not spill over from forest fragments to maize fields in a landscape mosaic in central Argentina

Marco Ferrante\textsuperscript{a*}, Ezequiel González\textsuperscript{b}, Gabor L. Lövei\textsuperscript{b}

\textsuperscript{a}Aarhus University, Department of Agroecology,
Flakkebjerg Research Centre, Forgøsvej 1, DK-4200 Slagelse, Denmark

E-mail: Marco.Ferrante@agro.au.dk

Phone: +45 50229827

\textsuperscript{b}E. González
Centro de Investigaciones Entomológicas de Córdoba,
Instituto Multidisciplinario de Biología Vegetal,
Universidad Nacional de Córdoba, CONICET. Córdoba, Argentina.

E-mail: ezenofx@gmail.com

*Corresponding author
Abstract

South America is undergoing a rapid and large scale conversion of natural habitats to cultivated land. Ecosystem services (ESs) still remain important but their level and sustainability are not known. We quantified the intensity of natural pest control in a South American agricultural landscape containing remnants of the original chaco serrano forest by using the artificial sentinel prey method. We sought to identify the main predators, and the effect of landscape configuration and maize phenology on predation pressure by invertebrate and vertebrate predators in this recently created landscape. The most common predators were chewing insects (50.4% predation events), birds (22.7%), and ants (17.5%). Overall predation rates in forest fragments (41.6% d⁻¹) were significantly higher than in the surrounding maize fields (21.5% d⁻¹). Invertebrate predation was higher inside and at the edge of forest fragments than within fields, and did not change with increasing distance from a fragment edge, indicating a lack of spillover from the native habitat remnants to the cultivated matrix at the local scale. Overall predation pressure declined with increasing distance from a near source area, but distance from a continuous forest had a positive impact on predation by invertebrates and a negative impact on vertebrate predation.

Keywords: biological control; chaco serrano; ecosystem services; edge effect; fragmentation; sentinel prey
Introduction

With the increasing size of the human population, demand on various resources has accelerated dramatically (Steffen et al., 2015). This “Great Acceleration” has also had impacts on the various large-scale ecological processes that form the basis of ecosystem services (ESs) (de Groot et al., 2002), on which sustainable agriculture depends (Tilman et al., 2002). Because biodiversity provides ESs, the two concepts are not always separated (Mace et al., 2012), although neither of them should be used as proxy for the other. Current agricultural production faces a serious challenge due to depending on massive non-renewable external inputs (Gliessman, 2015).

Increased reliance on ESs for sustainable agricultural production is inevitable. This situation brings up important global challenges: 1.) to quantify the intensity of, as well as track and directly monitor changes in ESs, 2.) to identify the effect of agricultural management practices on ESs, and 3.) to develop landscapes that sustain ESs (Tscharntke et al., 2005).

Conversion of natural areas to agriculture remains among the major drivers of biodiversity loss (Fahrig, 2003; Tscharntke et al., 2012). Such conversion generates landscapes consisting of a matrix of cultivated areas, in which natural habitat fragments varying in number, size and distance from each other are embedded (Fahrig, 2003). Traditionally, the remaining natural habitat fragments were considered refuges not only for native biodiversity, but also for species providing pest control (Bianchi et al., 2006), or pollination (Kremen et al., 2004); the matrix was seen unsuitable to sustain these populations (Simberloff & Abele, 1976). However, this turned out to be an oversimplification of a dynamic relationship between habitat fragments, edges, and matrix (Forman & Godron, 1981). Both the matrix (Kupfer et al., 2006) and the edges (Forman & Baudry, 1984; Magura et al., 2017) have great influence on the communities within the fragments. Individuals frequently move between these landscape elements (Blitzer et al., 2012;
González et al., 2016), and some species are closely related to the edges themselves (Duelli & Obrist, 2003; Lövei et al., 2006).

Natural habitats often increase the diversity and abundance of natural enemies (Bianchi et al., 2006; Chaplin-Kramer et al., 2011). Area (Fahrig, 2003), isolation (Kruess & Tscharntke, 2000), permeability (i.e. perimeter/area ratio, (Stoner & Joern, 2004; Wu, 2007)), and proximity to non-crop habitats (Clough et al., 2005; González et al., 2015; Tscharntke et al., 1998) influence arthropod densities and distribution, and their beneficial effects on crops. Temporal dynamics is also important, as movements of natural enemies between natural fragments and crops change in direction and intensity (Rand et al., 2006). Less is known about the effects of landscape structure on predation, particularly by generalist species (Chaplin-Kramer et al., 2011).

We sought to fill this gap by examining the relationship between predation pressure and landscape parameters in a recently converted, cultivated landscape in central Argentina. The original vegetation of this land is the chaco serrano, one of the most threatened subtropical habitats, as 94% of its original area has been recently converted to large-scale maize and soybean production (Zak et al., 2004). Earlier studies documented the biodiversity-supporting role of the remaining fragments (González et al., 2015; 2017a), and the movement of certain beneficial arthropods between forest remnants and the surrounding cultivated areas (González et al., 2016).

Specifically, we tested the following hypotheses:

H1: Predation pressure in forest fragments is higher than in their cultivated surroundings. We expected this because the forest fragments have higher primary production, larger standing biomass, and less disturbance than the crop, all of which can generate more food for herbivores,
thus indirectly also favouring predators, and cause higher predation pressure. Note that our
method cannot distinguish whether such higher predation pressure would emerge from higher
predator density, higher predator activity, or a combination of the two.

H2: Predation pressure in the edge is higher than either in the centre of the fragment, or in the
matrix. Natural enemies residing in edges may benefit from complementary resources from both
adjacent habitats (Ries et al., 2004), and reach higher densities there. Additionally, the edge also
can support a specific set of edge-preferring species (Duelli & Obrist, 2003), and the higher
predator diversity may increase predation pressure.

H3: Predation pressure is higher in fragments which are larger or closer to the supposed source
habitat, the non-converted, continuous forest, than in smaller fragments, or in those farther away
from these source habitats. In this landscape, larger fragments have higher densities of natural
enemies (González et al., 2015), and flying natural enemies move out of the forest fragments
more than into those (González et al., 2016). Moreover, predation pressure can be positively
correlated with edge density or perimeter amount, because there often are local density increases
at edges (Andrén, 1995).

H4: Predation pressure by invertebrate predators decreases with increasing distance from the
fragment edge, due to a decrease in densities or mobility of natural enemies that reside in the
forest fragment but move out to feed in the surrounding crop (spillover or halo, Blitzer et al.,
2012). While invertebrate predators can be affected by factors at small scales (Gaston &
Blackburn, 1996), we did not expect such gradient for vertebrate predators that have higher
mobility.
H5: Invertebrate predation pressure would be positively related to ground cover, because ground-active arthropods prefer vegetation or litter against bare ground (Koivula et al., 1999; Magura, 2002), and have higher densities in such patches.

H6: We also hypothesised that predation reaches its peak during maize flowering, as a consequence of increased predator densities at this time. This could happen either because these natural enemies consume pollen themselves, or because they are attracted to the field by the increased density of other, pollen-feeding arthropods (Pilcher et al., 2005).

We found predation rates up to 42% d\(^{-1}\), constituting strong top-down effects in this landscape. There was qualified support for our hypotheses: invertebrate but not vertebrate predation rates were significantly higher within the forest fragments and along the edges than within the crop. Ground cover increased predation pressure but only in the maize fields. Contrary to expectations, distance from the continuous forest was positively related to invertebrate, while negatively to vertebrate predation pressure, indicating that vertebrate and invertebrate predators perceive the same landscape differently.
Material and methods

Study site

Our study site was located in Córdoba Province (31.10°–31.30°S and 64°–64.30°W) in central Argentina. The original vegetation of the study area is chaco serrano, the southern part of a seasonally dry forest, gran chaco, with *Aspidosperma quebracho-blanco* and *Schinopsis quebracho* forming the canopy, and a slightly shorter subcanopy made up of several leguminous species. There is a scrub-like shrub and herbaceous layer. Currently, chaco forest is restricted to fragments of varying size in a cultivated landscape; the larger patches cover terrain unsuitable for large-scale, mechanised agriculture typical of the region (Nanni & Grau, 2014). The dominant crop in the region is maize (*Zea mays*), an important crop in Argentina (planted on almost 5 million ha, FAOStat, 2017). In this landscape, we selected eleven forest fragments (Table S1), with sizes ranging from 0.5 ha to 15 ha. On each fragment, we took the following measurements from Google Earth Images (https://www.google.com/earth/):

1. fragment size (ha), fragment perimeter length (m), edge density (“ED”, calculated as the ratio of fragment perimeter and area; (Helzer & Jelinski, 1999));
2. degree of isolation. Various measures of isolation were calculated: the shortest distance from the nearest neighbouring forest fragment (“Isolation 1”) (Krebs, 1989), the shortest distance from the sampled edge of a fragment to the next one (“Isolation 2”), the shortest distance between the given forest fragment and the nearest edge of the continuous, native forest (“Isolation 3”), and the shortest distance between the forest fragment and the continuous native forest by a “stepping stone” process of dispersal (“Isolation 4” see example in Fig. S1). However, Isolation 3 and
Isolation 4 were highly correlated, an in order to avoid multicollinearity we only used the simplest measurement, Isolation 3, for the analysis.

Measuring predation

During the southern summer (January-March) of 2016, we measured predation intensity at eight positions at each fragment: in the interior (> 15m from the edge), at the edge (defined as the transitional, uncultivated area between the forest fragment and the maize field), and at 1m, 2m, 5m, 10m, 20m and 40m from the edge into the maize field. We used artificial caterpillars (15mm long, 3mm diam.) made of green plasticine (Smeedi plus, V. nr. 776609, Denmark; Howe et al., 2009). To minimise the risk of damaging the caterpillars during handling, they were glued individually on small pieces of reed and transported to the field in glass tubes. At each position, we placed five caterpillars at 1m distance from each other, giving a total of 40 caterpillars per site. Sentinel prey were placed in the shadow to avoid damage by direct sunlight in the morning, and were left exposed to predators for 24h. The following day they were inspected in the field for signs of predation, using a hand-held magnifying glass (20x). If necessary, caterpillars were transported to the laboratory for verification and photographing. Signs of predation were identified from photographs in published papers (Ferrante et al., 2014; Low et al., 2014). Six sampling sessions were made, starting on 14 January 2016, when maize was approximately 16cm tall (BBCH phenological stage 15-16, (Lancashire et al., 1991)), and finishing on 28 March 2016, when maize was at milky ripening (development stage 89). In total, 2600 artificial caterpillars were exposed, of which 30 (1.15%) were lost. The largest fragment only had five sessions (no prey exposed on 14 January 2016).
Habitat characterisation

At every sampling location, we photographed two different areas on the soil surface, each of 25 cm x 50 cm, identified with the help of a metal frame. From these photos, we calculated the area of bare ground (“BareGround”, in %), as well as the area covered by live (“LivePlant”, in %) and dead plant (“DryGround”, in %) material, using the program ImageJ. For evaluation, we used the mean values measured on the two photo frames per position.

Data analysis

In order to test which landscape factors influence predation intensity, we used a multi-model information-theoretic (IT) approach (Burnham & Anderson, 2003). Such approach consists in specifying a set of candidate models based on a priori knowledge or specific hypotheses, ranking the models from the lowest to the highest AIC (Akaike, 1998) and Akaike weight (AICw) (Burnham & Anderson, 2003), and averaging all the models with ∆AIC < 2 or AICw ≥ 0.9. Models which do not fit such criteria lack sufficient support and are discarded (Burnham & Anderson, 2003). The IT approach is suitable for complex analyses which include many models, and compared to the traditional null-hypothesis testing for the model variable it has the advantage of evaluating the support for each model simultaneously, and reducing model uncertainty by averaging the most reliable models (Zuur et al., 2009). Before specifying the models, we graphically tested each numerical factor for outliers using boxplots and dot charts, and for collinearity between factors using the Variance Inflation Factor (VIF) (Ieno & Zuur, 2015). We did not found outliers, but there was collinearity between Position and BareGround, and between Area, Perimeter and ED.
To systematically address our different hypotheses, we separately analysed total predation, and predation attributed to invertebrates, vertebrates, chewing insects (excluding ants), ants, birds, and small mammals. The candidate model sets were defined avoiding collinear factors, and accounted for 93 models for total predation and each of the invertebrate predators, and 47 models for each of the vertebrate ones. Each set included models with a single factor, all the possible additive models with two factors, and all the possible additive models with two factors plus phenology. Site was always considered a random factor, while phenology was a random factor only in models which did not include it already as a fixed factor. We did not include other models with interactions as we did not have any specific a priori hypothesis for them. From the set of models of vertebrate predators, we also excluded models including BareGround, as we did not expect it to be relevant for them. When examining factors influencing predation by vertebrates, position was coded as “forest”, “edge”, and “maize field”, without considering different distances within the crop, which are likely to be too small for predators with high mobility. For each set, we identified the best models, and the estimates of these were averaged to obtain the final model (Burnham & Anderson, 2003). Tukey’s post-hoc t-test was used to identify significant differences in predation intensity for categorical variables (Phenology and Position). The statistical analysis was performed with the statistical program R, version 3.3.1 (R Core Team, 2016). The generalized linear mixed models were created using the package “lme4” (Bates et al., 2014), the supported models averaged using the package “MuMIn” (Barton et al., 2016), and the post-hoc Tukey t-test was performed using the package “multcomp” (Hothorn et al., 2008).
Results

Predation pressure

Totally, 692 artificial caterpillars were attacked, giving an overall median predation rate of 27% d\(^{-1}\) (range = 21.8-32.9% d\(^{-1}\), n = 11, Table 1). Four predator groups were identified: chewing insects (50.4% of all predation), and ants (17.5%) as invertebrate predators; birds (22.7%), and small mammals (10.0%) as vertebrate predators. Unknown predators accounted for 1.7% of the artificial caterpillars attacked. Within the maize field, the highest predation was found at 40m from the edge (median = 26.7% d\(^{-1}\), range = 6.9-36.7% d\(^{-1}\), n = 11). Chewing insect predation was highest at 1m (mean = 10.8% d\(^{-1}\), SD = 6.4, n = 11), ant predation at 40m (mean = 3.4% d\(^{-1}\), SD = 3.4, n = 11), bird predation at 20m (mean = 11.0% d\(^{-1}\), SD = 5.5% d\(^{-1}\), n = 11), and small mammal at 10m from the forest edge (mean % = 1.6% d\(^{-1}\), SD = 2.4% d\(^{-1}\), n = 11).

Except for total invertebrate predation, all predator groups had more than one model with the lowest AIC values, indicating the need for model averaging (Table 2). Two or three variables in each model were important to explain the observed trends in predation pressure, with maize phenology as the most frequent factor, while isolation measures and habitat were also relevant for most predators.

H1: Predation pressure in forest fragments higher than in cultivated habitats

Total predation rates within forest fragments (mean = 41.6% d\(^{-1}\), SD = 12.5% d\(^{-1}\), n = 11) were significantly higher (Tukey’s t-test, p < 0.001 for all comparisons) than in the maize fields, at any distance from the forest (mean = 21.5% d\(^{-1}\), SD = 3.2% d\(^{-1}\), n = 11).
The same was found for invertebrate predation rates (mean\textsubscript{forest} = 34.7\%d\textsuperscript{-1}, SD = 15.3\%d\textsuperscript{-1}, n = 11; mean\textsubscript{crop} = 12.6\%d\textsuperscript{-1}, SD = 3.6\%d\textsuperscript{-1}, n = 11; Tukey’s t-test, p < 0.001 for all comparisons), as well as (for ant predation (mean\textsubscript{forest} = 34.4\%d\textsuperscript{-1}, SD = 15\%d\textsuperscript{-1}, n = 11; mean\textsubscript{crop} = 12.6\%d\textsuperscript{-1}, SD = 3.6\%d\textsuperscript{-1}, n = 11; Tukey’s t-test, p < 0.001 for all comparisons). Chewing insect predation was not significantly affected by the habitat.

Vertebrate predation within the forest fragments (mean = 6.9\%d\textsuperscript{-1}, SD = 6.5\%d\textsuperscript{-1}, n = 11) was not significantly different than within the crop (mean = 8.6\%d\textsuperscript{-1}, SD = 3.3\%d\textsuperscript{-1}, n = 11). Bird predation within the forest fragments (mean = 0.3\%d\textsuperscript{-1}, SD = 1\%d\textsuperscript{-1}, n = 11) was significantly lower (Tukey’s t-test, p < 0.01) than within the crop (mean = 7.9\%d\textsuperscript{-1}, SD = 3\%d\textsuperscript{-1}, n = 11), while the opposite was registered for mammal predation (mean\textsubscript{forest} = 6.6\%d\textsuperscript{-1}, SD = 6\%d\textsuperscript{-1}, n = 11, mean\textsubscript{crop} = 0.74\%d\textsuperscript{-1}, SD = 0.79\%d\textsuperscript{-1}, n = 11; Tukey’s t-test, p < 0.001).

H2: Predation pressure along forest edges higher than in the cultivated matrix or the centre of the fragment

Overall predation rates along the edges of forest fragments (mean = 44.5\%d\textsuperscript{-1}, SD = 11.1\%d\textsuperscript{-1}, n = 11) were not higher than within forest fragments, but were significantly higher (Tukey’s t-test, p < 0.001 for all comparisons) than within the crop (mean = 21.5\%d\textsuperscript{-1}, SD = 3.2\%d\textsuperscript{-1}, n = 11).

Similarly, invertebrate predation along edges (mean = 33.1\%d\textsuperscript{-1}, SD = 11.6\%d\textsuperscript{-1}, n = 11) was not significantly different than within the forest fragments, but was significantly higher (Tukey’s t-test, p < 0.001 for all comparisons) than within the crop (mean = 12.6\%d\textsuperscript{-1}, SD = 3.6\%d\textsuperscript{-1}, n = 11). Ant predation at edges (mean = 33.1\%d\textsuperscript{-1}, SD = 11.6\%d\textsuperscript{-1}, n = 11) was significantly higher (Tukey’s t-test, p < 0.05) than predation at 10m from the forest edge (mean = 0.9\%d\textsuperscript{-1}, SD = ...
2.2% d^{-1}, n = 11), but not at other distances. Chewing insect predation was not significantly
affected.

Vertebrate predation along forest edges (mean = 14.8% d^{-1}, SD = 12.8% d^{-1}, n = 11) was not
significantly higher than in the crop (mean = 8.6% d^{-1}, SD = 3.3% d^{-1}, n = 11), or within the forest
fragments (mean = 6.9% d^{-1}, SD = 6.5% d^{-1}, n = 11). Bird predation rate along forest edges (mean
= 1.5% d^{-1}, SD = 3.1% d^{-1}, n = 11) was not significantly different from predation inside the forest
fragments (mean = 0.3% d^{-1}, SD = 1% d^{-1}, n = 11) but significantly lower (Tukey’s t-test, p <
0.001) than in the crop (mean = 7.8% d^{-1}, SD = 3% d^{-1}, n = 11). Mammal predation at edges
(mean = 10.7% d^{-1}, SD = 12.4% d^{-1}, n = 11) was significantly higher (Tukey’s t-test, p < 0.001)
than in the crop (mean = 0.74% d^{-1}, SD = 0.79% d^{-1}, n = 11) but not higher than in the fragments
(mean = 6.6% d^{-1}, SD = 6% d^{-1}, n = 11).

H3: Predation pressure higher in larger fragments or closer to the source habitat

Fragment area had a significantly positive effect only on mammal predation (GLMM, z = 4.78, p
< 0.001). On predation by chewing insects, contrary to the hypothesis, it had a significant
negative effect (GLMM, z = 2.56, p < 0.05).

Distance from the closest neighbouring fragment (Isolation 1) had a significant positive effect on
total (GLMM, z = 2.49, p < 0.05), vertebrate (GLMM, z = 3.36, p < 0.001), bird (GLMM, z =
2.77, p < 0.01) predation rates, and a marginally positive effect on predation by ants (GLMM, z
= 1.68, p < 0.1).
Distance from the closest neighbouring fragment at the sampled edge (Isolation 2) had a significant negative effect on mammal predation (GLMM, \(z = 4.73, p < 0.001\)).

Distance from the continuous forest (Isolation 3) had a significant positive effect on predation by all invertebrates (GLMM, \(z = 3.62, p < 0.001\)), and chewing insects (GLMM, \(z = 2.79, p < 0.01\)), but a negative effect on vertebrate predation (GLMM, \(z = 4.66, p < 0.001\)).

Edge density did not affect predation by any group, while fragment perimeter had a significant negative effect on predation by chewing insects (GLMM, \(z = 2.16, p < 0.05\)).

**H4:** Predation pressure by invertebrate predators decreases away from the forest edge

Distance from the forest edge did not significantly affect predation rates.

**H5:** Predation pressure by invertebrate predators positively related to ground cover

Total and chewing insect predation rates were significantly (GLMM, \(z = 9.97, p < 0.001\) and \(z = 8.77, p < 0.001\)) positively related to live plant cover, but the same was not true for invertebrate and ant predation rates. Other elements of surface cover (amount of dead plant material or bare ground) had no influence on predation rates by any group.

**H6:** Predation pressure peaks during maize flowering

Phenology had a significant influence on predation rates by all identified predator groups but mammals (Table 3). Total predation during the early milky ripening stage (mean = 37.8\%d\(^{-1}\), SD = 9\%d\(^{-1}\), \(n = 11\)) was significantly higher than any other phases (Tukey’s \(t\)-test, \(p < 0.001-0.05\)) excluding maize ripening, which was only marginally significant (Tukey’s \(t\)-test, \(p < 0.1\)).
Invertebrate predation rates during the early milky ripening stage (BBCH code 73, late February) had an average of 31.1% d⁻¹ (SD = 10.4% d⁻¹, n = 11), significantly higher than at any other phases (Tukey’s t-test, p < 0.001 for all comparisons). Moreover, invertebrate predation during the milky ripening stage (mean = 19.1% d⁻¹, SD = 7.6% d⁻¹, n = 11) and at cob ripening (mean = 15.2% d⁻¹, SD = 5.4% d⁻¹, n = 11) were significantly higher (Tukey t-test, p < 0.05 for both) than during late January (BBCH code 17-18) (mean = 8.6% d⁻¹, SD = 4.5% d⁻¹, n = 11). Ant predation was significantly (Tukey t-test, p < 0.01) higher in early January (BBCH code 15-16) (mean = 7.7% d⁻¹, SD = 3.4% d⁻¹, n = 11) than in late January, and marginally significantly higher (Tukey t-test, < 0.1) than during milk stage (BBCH code 77) (mean = 3.4% d⁻¹, SD = 2.0% d⁻¹, n = 11). Predation by chewing insects was also significantly (Tukey t-test, p < 0.001) higher at the early milky ripening stage (mean = 27.4% d⁻¹, SD = 10.6% d⁻¹, n = 11) than other phenological phases.

Vertebrate predation peaked during ripening at 14.0% d⁻¹ (SD = 10.0% d⁻¹, n = 11). This was significantly higher than any of the other sampling occasion (Tukey t-test, p < 0.05), except during early January (Tukey t-test, p < 0.1), and during milky ripening (BBCH code 77). Bird predation was significantly higher (Tukey t-test, p < 0.001-0.05) when at cob ripening (BBCH code 89) (mean = 11.5% d⁻¹, SD = 9.3% d⁻¹, n = 11) than at other times. Mammal predation peaked at maize flowering (BBCH code 67) (mean = 3.9% d⁻¹, SD = 2.6% d⁻¹, n = 11).
Discussion

Overall, we registered high predation pressure on the artificial caterpillars: nearly half of them were attacked within 24h in chaco serrano forest fragments. This is among the higher values recorded so far world-wide (Lövei & Ferrante 2017). Currently, we have few data from cultivated fields (but see Howe et al., 2015, Barbaro et al., 2017), and no published studies from maize fields from anywhere, making direct comparisons impossible. The ground level predation rate on artificial caterpillars found here was lower than in winter wheat in Denmark, and unsurprisingly, the relative contribution of the predatory groups responsible for the attacks was different: bird and ant predation rates were much higher in Argentina than in Denmark (Mansion-Vaquié et al., 2017). These differences exist possibly due to the positive effect of landscape heterogeneity on farmland birds (Smith et al., 2010), and to the great ant abundance in subtropical areas (Hölldobler & Wilson, 1990), respectively.

Concerning the impact of landscape, total predation, as well as predation by invertebrates, ants, chewing insects, and mammals were higher in forest fragments than in the crop, supporting our hypothesis 1. The same was not true for vertebrate and bird predation rates. It is plausible that habitat complexity plays a role for invertebrate predation rates, as well as it does for invertebrate predator abundance (Langellotto & Denno, 2004). The difference in bird and mammal predation rates could result because dense vegetation makes these habitats less accessible for birds, while more attractive for mammals as they have a lower predation risk in habitats with taller vegetation (Doherty et al., 2015). The registered predation pressure for all predators (except birds) was higher along edges than within the crop, but never significantly higher than within the forest fragments (H2 rejected). This indicates that forest fragment-living predators regularly visited the edge, or that the edge supported a suit of predators that exerted predation pressure similar to the
inner parts of a forest fragment. In this landscape, habitat complexity may be more important
than complementarity of resources, because in addition to prey, invertebrate predators need
favourable microclimatic conditions, and refuges (Langellotto & Denno, 2004).

Bigger fragments had higher predation rates by mammals, but lower ones by chewing insects
(H3 partially supported). Negative relationships between invertebrate abundance and habitat area
have been previously reported for a coccinellid predator (Elliott et al., 2002) and ground-
dwelling insect predators were more abundant in small than big patches of chaco serrano
(Moreno et al., 2013). This becomes interpretable if we consider that small mammals are also
predators of carabids, spiders, and other chewing insects. Small mammals may plausibly need
bigger fragments to sustain populations where they exert a higher predation pressure on
invertebrate prey – so chewing insects will, by corollary, become less abundant there than in
smaller fragments. Such antagonism between carabids and small mammals is documented (Lövei
& Sunderland, 1996) and was experimentally proven in semi-arid habitats in North America
(Parmenter & MacMahon, 1988).

Small mammal predation also decreased as distance from the assumed closest source area
increased, but the opposite was found for predation by birds. Difference in mobility may explain
why mammal but not bird predation rates were so affected by short distances.

Invertebrate and chewing insect predation rates increased with increasing distance from the
continuous forest which was probably scale-dependent. The mean distance of our fragments to
the continuous source forest was 4.5 km, which may be too far to allow for regular movements
between source and fragments for these invertebrates. These fragments have possibly become an
“independent set of islands” with their own dynamics, and they no longer depended on the
source. The opposite pattern was found for vertebrate predation rates, which suggest that vertebrate predators depended on these areas. Large and continuous forests frequently sustain large populations (Andrén, 1994; Pardini et al., 2005; Uezu et al., 2005) and can therefore be sources of individuals for nearby patches. Birds in the chaco serrano move actively between fragments (Díaz Vélez et al., 2015). Moreover, the effect of isolation is highly influenced by the quality of the matrix (Prugh et al., 2008). In our case, an important factor could be the low level of disturbance in this cultivation system: the maize fields in this area are very big, and between sowing and harvest, are very rarely disturbed by humans, and this encourages the movement of birds.

We found no positive effect of the proximity to the forest edge to invertebrate predation (H4 rejected). This suggests that there was little spillover of invertebrate predators from the forest or only a part of the predators present in the forest edge moved into the matrix (Duelli & Obrist, 2003). Therefore, these forest fragments can act as sources of flying natural enemies to adjacent crops (González et al., 2015, 2017b), but not of ground-dwelling predators.

We found a positive correlation between total and chewing insect predation rates and live plant ground cover (H5 supported). Ground cover is important for soil-surface-active arthropods (Magura et al., 2002), which are probably a key group attacking artificial caterpillars (Ferrante et al., 2014; Mansion-Vaquí et al., 2017), and which are abundant in chaco serrano (E. Gonzaléz, pers obs.). Plant material also affects soil pH, humidity and organic content (Sadler et al., 2006), and it is usually preferred by invertebrates to bare ground.

Crop phenology was an important factor for both invertebrate and vertebrate predators. Total predation, invertebrate, and chewing insect predation rates were higher during the early milky
ripening stage, vertebrate and bird predation rates reached their peak at cob ripening, and
mammal predation rate was highest at maize flowering (H6 partially supported). The invertebrate
predation rate peak may be explained by omnivorous predators using the most abundant resource
available (i.e. pollen during flowering and prey after the end of it), as observed with the
coccinellid *Coleomegilla maculata* in maize crops (Lundgren et al., 2004). Moreover, predatory
insects not consuming pollen may show a delay, being attracted to the crop only when the prey
density is already high (May, 1973), in our case at the phenological stage immediately after
flowering. Small mammals may respond to an increase in prey faster than invertebrate predators
as they have highly dynamic landscape occupancy patterns (Brunner et al., 2013).

This study was the first application of the artificial caterpillars in Argentina, as well as in the
chaco serrano. The scales at which different predators perceive the landscape, the relative
permeability of the matrix, and predator mobility, can explain the patterns we observed.

Landscape heterogeneity does not only support biodiversity in agro-environment (Benton et al.,
2003), but also ESs such as biological control. The relationship between biodiversity and ESs is
complex and can be described by various possible models (Tscharntke et al., 2005). Using direct
measurements of ecosystem functioning, rather than “estimating” them by indirect measures,
would help to articulate this complex relationship. Our results indicated that both invertebrate
and vertebrate predation rates can be affected by the same factor in different ways. The use of
artificial caterpillars, which allows partitioning total predation rates to various predator groups,
seems particularly suitable in such cases, and we encourage their wider use to understand factors
influencing predation pressure in various habitats.
Acknowledgements

We thank Florencia Castillo, Guillermo Flores, Gustavo Rojo and Juan Finello for help during fieldwork, the Estancia Santo Domingo, Rio Ceballos for permission to use their land, Eduardo Trumper and the late Julio Edelstein for support. This is publication no. 16 of the AMIGA project, supported by the European Community, Framework 7 program, contract no. n° 289706, and is in partial fulfilment of the PhD requirements at Aarhus University (MF).

Author contributions: MF, EG and GL designed the study, field work was done by MF and EG, data analysis and writing shared by all authors.
References


Brunner, J. L., Duerr, S., Keesing, F., Killilea, M., Vuong, H., & Ostfeld, R. S. (2013). An experimental test of competition among mice, chipmunks, and squirrels in deciduous forest fragments. *PLOS One, 8*(6), e66798.


Table 1. The number of artificial caterpillars attacked by various predators at Rio Ceballos, Córdoba, Argentina, during the southern summer of 2015/2016. Caterpillars were placed at various positions in forest fragments and the surrounding maize fields. Multiple attacks by the same predator were counted as single attack, but attacks by different predators were considered independent.

<table>
<thead>
<tr>
<th>Position</th>
<th>No. of caterpillars exposed</th>
<th>No. of caterpillars attacked by</th>
<th>Chewing insects*</th>
<th>Ants</th>
<th>Birds</th>
<th>Mammals</th>
<th>Unknown predators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest</td>
<td>324</td>
<td>66</td>
<td>52</td>
<td>1</td>
<td>21</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Edge</td>
<td>322</td>
<td>86</td>
<td>22</td>
<td>5</td>
<td>34</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1m from edge</td>
<td>322</td>
<td>35</td>
<td>10</td>
<td>17</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>2m from edge</td>
<td>319</td>
<td>26</td>
<td>8</td>
<td>27</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>5m from edge</td>
<td>323</td>
<td>42</td>
<td>9</td>
<td>20</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>10m from edge</td>
<td>319</td>
<td>34</td>
<td>3</td>
<td>21</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>20m from edge</td>
<td>324</td>
<td>26</td>
<td>6</td>
<td>36</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40m from edge</td>
<td>317</td>
<td>34</td>
<td>11</td>
<td>30</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>2570</td>
<td>349</td>
<td>121</td>
<td>157</td>
<td>69</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

*Excluding ants
Table 2. A list of the best models for explaining predation rates by various predator groups at Rio Ceballos, Cordoba, Argentina, during the southern summer of 2015/2016, based on ΔAIC and model weight.

<table>
<thead>
<tr>
<th>Predator group</th>
<th>Best models</th>
<th>AIC</th>
<th>ΔAIC</th>
<th>df</th>
<th>Model weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>All predators</td>
<td>Isolation1 + Phenology + Distance + (1</td>
<td>Site)*</td>
<td>2841.6</td>
<td>0.0</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Isolation1 + Phenology + LivePlant + (1</td>
<td>Site)</td>
<td>2842.5</td>
<td>0.8</td>
<td>9</td>
</tr>
<tr>
<td>Invertebrates</td>
<td>Isolation3 + Phenology + Distance + (1</td>
<td>Site)</td>
<td>2209.3</td>
<td>0.0</td>
<td>15</td>
</tr>
<tr>
<td>Chewing insects**</td>
<td>Isolation3 + Phenology + LivePlant + (1</td>
<td>Site)</td>
<td>1867.1</td>
<td>0.0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Area + Phenology + LivePlant + (1</td>
<td>Site)</td>
<td>1868.4</td>
<td>1.3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Perimeter + Phenology + LivePlant + (1</td>
<td>Site)</td>
<td>1869.8</td>
<td>2.7</td>
<td>9</td>
</tr>
<tr>
<td>Ants</td>
<td>Isolation1 + Phenology + Distance + (1</td>
<td>Site)</td>
<td>890.5</td>
<td>0.0</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Distance + Phenology + (1</td>
<td>Site)</td>
<td>891.1</td>
<td>0.6</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Isolation3 + Phenology + Distance + (1</td>
<td>Site)</td>
<td>892.0</td>
<td>1.4</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Isolation2 + Phenology + Distance + (1</td>
<td>Site)</td>
<td>892.1</td>
<td>1.6</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Area + Phenology + Distance + (1</td>
<td>Site)</td>
<td>892.5</td>
<td>1.9</td>
<td>15</td>
</tr>
<tr>
<td>Vertebrates</td>
<td>Isolation3 + Phenology + Isolation1 + (1</td>
<td>Site)</td>
<td>1500.1</td>
<td>0.0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Isolation3 + Isolation1 + (1</td>
<td>Phenology) + (1</td>
<td>Site)</td>
<td>1504.0</td>
<td>3.9</td>
</tr>
<tr>
<td>Birds</td>
<td>Habitat + Phenology + Isolation1 + (1</td>
<td>Site)</td>
<td>1104.2</td>
<td>0.0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Habitat + Phenology + (1</td>
<td>Site)</td>
<td>1107.6</td>
<td>3.4</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Habitat + Phenology + Isolation3 + (1</td>
<td>Site)</td>
<td>1107.6</td>
<td>3.4</td>
<td>10</td>
</tr>
<tr>
<td>Mammals</td>
<td>Area + Habitat + (1</td>
<td>Phenology) + (1</td>
<td>Site)</td>
<td>524.3</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Habitat + Phenology + Isolation2 + (1</td>
<td>Site)</td>
<td>527.6</td>
<td>3.3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Habitat + Phenology + Area + (1</td>
<td>Site)</td>
<td>528.4</td>
<td>4.1</td>
<td>10</td>
</tr>
</tbody>
</table>

*Factors in parenthesis are considered random factors.

**Excluding ants
Table 3. Effects of the landscape variables on the seven final averaged models. Arrows indicate positive (↑) or negative (↓) effect of a numerical variable, while symbols indicate significance levels (+ = p<0.1; *= p<0.05; **=p<0.01,***= p<0.001). Only variables with at least one significant value are shown.

<table>
<thead>
<tr>
<th></th>
<th>Total predation</th>
<th>Invertebrates</th>
<th>Chewing insects</th>
<th>Ants</th>
<th>Vertebrates</th>
<th>Birds</th>
<th>Mammals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>↓*</td>
<td>↓*</td>
<td>+</td>
<td></td>
<td>↑***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolation1</td>
<td>↑*</td>
<td>↑*</td>
<td>↑**</td>
<td>↑**</td>
<td>↑**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolation2</td>
<td></td>
<td>+</td>
<td>↓*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolation3</td>
<td>↑**</td>
<td>↑**</td>
<td>+</td>
<td>↓***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenology</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Distance</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habitat</td>
<td></td>
<td>*</td>
<td>**</td>
<td>***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LivePlant</td>
<td>↑***</td>
<td></td>
<td>↑***</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Edge density, fragment perimeter, % coverage by dead plant material or bare soil were not significant for any predator group.
Figure caption

Figure 1 - Mean% daily predation (±SD) at the 11 sites, within the forest fragments, along the edges, and within the maize field, at Rio Ceballos, Córdoba, Argentina, during the southern summer of 2015/2016.

Supplementary material captions

Figure S1- Map of the study sites at Rio Ceballos, Córdoba, Argentina. The four measures of isolation are illustrated for fragment no. 9. Slashed line marks the shortest distance from the nearest neighbouring forest fragment (“Isolation 1”, in this case to fragment no. 8); a dotted line shows the shortest distance from the sampled edge of a fragment to the next one (“Isolation 2”; here to fragment no. 6); slash/dotted line marks the shortest linear distance to the nearest edge of the continuous forest (“Isolation 3”); solid lines show the shortest “stepping stone” distances to the continuous forest (“Isolation 4”).

Table S1. Landscape variables for each of the experimental forest fragment sites at Rio Ceballos, Córdoba, Argentina, during the southern summer of 2015/2016.

Table S2. Output of the final averaged models for predation rates on artificial caterpillars by all predators, and for each identified predator group at Rio Ceballos, Córdoba, Argentina, during the southern summer of 2015/2016. Factors under individual predator groups are ranked by their $z$ values.
Figure 1 - Mean% daily predation (±SD) at the 11 sites, within the forest fragments, along the edges, and within the maize field, at Rio Ceballos, Córdoba, Argentina, during the southern summer of 2015/2016.
Supplementary material to the article Predators do not spill over from forest fragments to maize fields in a landscape mosaic in central Argentina by Marco Ferrante, Ezequiel González, Gabor L. Lövei
Fig.S1- Map of the study sites at Rio Ceballos, Córdoba, Argentina. The four measures of isolation are illustrated for fragment no. 9. Slashed line marks the shortest distance from the nearest neighbouring forest fragment (“Isolation 1”, in this case to fragment no. 8); a dotted line shows the shortest distance from the sampled edge of a fragment to the next one (“Isolation 2”; here to fragment no. 6); slash/dotted line marks the shortest linear distance to the nearest edge of the continuous forest (“Isolation 3”); solid lines show the shortest “stepping stone” distances to the continuous forest (“Isolation 4”).
Table S1. Landscape variables for each of the experimental forest fragment sites at Rio Ceballos, Córdoba, Argentina, during the southern summer of 2015/2016.

<table>
<thead>
<tr>
<th>Site</th>
<th>Area (ha)</th>
<th>Perimeter (m)</th>
<th>Edge density</th>
<th>Isolation1 (m)</th>
<th>Isolation2 (m)</th>
<th>Isolation3 (m)</th>
<th>Isolation4 (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.6</td>
<td>605</td>
<td>378.1</td>
<td>10</td>
<td>163</td>
<td>1450</td>
<td>594</td>
</tr>
<tr>
<td>2</td>
<td>3.9</td>
<td>1014</td>
<td>260.0</td>
<td>93</td>
<td>162</td>
<td>1510</td>
<td>633</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>439</td>
<td>439.0</td>
<td>26</td>
<td>280</td>
<td>1650</td>
<td>704</td>
</tr>
<tr>
<td>4</td>
<td>8.9</td>
<td>1789</td>
<td>201.0</td>
<td>87</td>
<td>168</td>
<td>1880</td>
<td>764</td>
</tr>
<tr>
<td>5</td>
<td>4.8</td>
<td>1208</td>
<td>251.7</td>
<td>24</td>
<td>202</td>
<td>1430</td>
<td>524</td>
</tr>
<tr>
<td>6</td>
<td>12.0</td>
<td>2483</td>
<td>206.9</td>
<td>16</td>
<td>196</td>
<td>832</td>
<td>199</td>
</tr>
<tr>
<td>7</td>
<td>0.5</td>
<td>273</td>
<td>546.0</td>
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<td>212</td>
<td>1110</td>
<td>261</td>
</tr>
<tr>
<td>8</td>
<td>0.7</td>
<td>335</td>
<td>478.6</td>
<td>120</td>
<td>250</td>
<td>1280</td>
<td>427</td>
</tr>
<tr>
<td>9</td>
<td>3.6</td>
<td>741</td>
<td>205.8</td>
<td>122</td>
<td>234</td>
<td>1140</td>
<td>322</td>
</tr>
<tr>
<td>10</td>
<td>4.1</td>
<td>1050</td>
<td>256.1</td>
<td>25</td>
<td>289</td>
<td>999</td>
<td>359</td>
</tr>
<tr>
<td>11</td>
<td>15</td>
<td>2485</td>
<td>165.7</td>
<td>19</td>
<td>80</td>
<td>144</td>
<td>144</td>
</tr>
</tbody>
</table>
Table S2. Output of the final averaged models for predation rates on artificial caterpillars by all predators, and for each identified predator group at Rio Ceballos, Córdoba, Argentina, during the southern summer of 2015/2016. Factors under individual predator groups are ranked by their $z$ values.

<table>
<thead>
<tr>
<th></th>
<th>Estimated value</th>
<th>S.E.</th>
<th>Adjusted S.E.</th>
<th>$z$ value</th>
<th>Significance, $p$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total predation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Intercept)</td>
<td>-1.175</td>
<td>0.491</td>
<td>0.491</td>
<td>2.395</td>
<td>0.017</td>
</tr>
<tr>
<td>Live Plant Cover</td>
<td>2.024</td>
<td>0.203</td>
<td>0.203</td>
<td>9.973</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Distance: 10m</td>
<td>-1.090</td>
<td>0.183</td>
<td>0.183</td>
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</tr>
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<td>Distance: 2m</td>
<td>-1.030</td>
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<td>0.181</td>
<td>5.681</td>
<td>&lt;0.0001</td>
</tr>
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<td>0.180</td>
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<td>&lt;0.0001</td>
</tr>
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<td>-0.981</td>
<td>0.179</td>
<td>0.180</td>
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</tr>
<tr>
<td>Distance: 5m</td>
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<td>0.177</td>
<td>5.150</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Phenology: Early Milky</td>
<td>0.826</td>
<td>0.161</td>
<td>0.162</td>
<td>5.112</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Distance: 40m</td>
<td>-0.837</td>
<td>0.176</td>
<td>0.176</td>
<td>4.749</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Phenology: Ripe</td>
<td>0.419</td>
<td>0.166</td>
<td>0.166</td>
<td>2.528</td>
<td>0.016</td>
</tr>
<tr>
<td>Isolation 1</td>
<td>2.767</td>
<td>1.112</td>
<td>1.113</td>
<td>2.488</td>
<td>0.013</td>
</tr>
<tr>
<td>Phenology: BBCH17-18</td>
<td>-0.435</td>
<td>0.184</td>
<td>0.184</td>
<td>2.369</td>
<td>0.018</td>
</tr>
<tr>
<td>Phenology: Flowering</td>
<td>0.380</td>
<td>0.166</td>
<td>0.166</td>
<td>2.290</td>
<td>0.022</td>
</tr>
<tr>
<td>Phenology: Milky</td>
<td>0.362</td>
<td>0.166</td>
<td>0.166</td>
<td>2.181</td>
<td>0.029</td>
</tr>
<tr>
<td>Distance: Edge</td>
<td>0.131</td>
<td>0.162</td>
<td>0.162</td>
<td>0.807</td>
<td>0.420</td>
</tr>
<tr>
<td><strong>Predation by all invertebrates</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(Intercept)</td>
<td>-1.581</td>
<td>0.256</td>
<td>n.a.</td>
<td>-6.176</td>
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<td>1.158</td>
<td>0.186</td>
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<tr>
<td>Isolation 4</td>
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<td>n.a.</td>
<td>3.623</td>
<td>0.0002</td>
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<tr>
<td>Phenology: Flowering</td>
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<td>0.196</td>
<td>n.a.</td>
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<td>Phenology: BBCH17-18</td>
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<td>n.a.</td>
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</tr>
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<td>0.204</td>
<td>n.a.</td>
<td>-5.932</td>
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<td>Distance: 1m</td>
<td>-1.228</td>
<td>0.204</td>
<td>n.a.</td>
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<td>&lt;0.0001</td>
</tr>
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<td>0.215</td>
<td>n.a.</td>
<td>-6.743</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Distance: 2m  -1.549  0.221  n.a.  -7.015  <0.0001  
Distance: 20m -1.637  0.225  n.a.  -7.285  <0.0001  

**Predation by chewing insects**

(Intercept)  -3.258  0.429  0.429  7.595  <0.0001  
Live Plant Cover  2.138  0.244  0.244  8.768  <0.0001  
Phenology: Early Milky  1.786  0.235  0.235  7.595  <0.0001  
Phenology: Milky  1.029  0.247  0.247  4.164  <0.0001  
Phenology: Flowering  0.919  0.250  0.250  3.680  0.0002  
Isolation  0.403  0.145  0.145  2.785  0.005  
Area  -0.036  0.014  0.014  2.555  0.011  
Perimeter  -0.191  0.088  0.088  2.164  0.030  
Phenology: Ripe  0.517  0.262  0.262  1.969  0.049  
Phenology: BBCH17-18  0.031  0.284  0.284  0.109  0.913  

**Predation by ants**

(Intercept)  -1.324  0.399  0.399  3.321  0.0008  
Distance: 20m  -2.346  0.440  0.440  5.328  <0.0001  
Distance: 2m  -2.032  0.390  0.390  5.207  <0.0001  
Distance: 5m  -1.923  0.372  0.372  5.169  <0.0001  
Distance: 1m  -1.813  0.357  0.357  5.080  <0.0001  
Distance: 10m  -3.033  0.600  0.600  5.053  <0.0001  
Distance: 40m  -1.685  0.344  0.344  4.899  <0.0001  
Distance: Edge  -0.979  0.270  0.270  3.624  0.0003  
Phenology: BBCH17-18  -1.304  0.380  0.380  3.430  0.0006  
Phenology: Milky  -0.870  0.334  0.334  2.606  0.009  
Phenology: Early Milky  -0.597  0.312  0.312  1.914  0.056  
Isolation1 4.277  2.54  2.54  1.683  0.092  
Phenology: Ripe  -0.444  0.300  0.300  1.479  0.139  
Isolation  0.303  0.284  0.284  1.067  0.286  
Phenology: Flowering  -0.299  0.291  0.291  1.026  0.305  
Isolation 2  2.130  2.146  2.147  0.992  0.321  
Area  -0.022  0.027  0.027  0.808  0.419  

**Predation by all vertebrates**

(Intercept)  -1.871  0.275  0.275  6.814  <0.0001  
Isolation  0.774  0.166  0.166  4.658  <0.0001  
Isolation  5.890  1.755  1.756  3.355  0.0007  
Phenology: Ripe  0.644  0.240  0.240  2.686  0.007  
Phenology: Early Milky  -0.190  0.275  0.275  0.691  0.490  

103
<table>
<thead>
<tr>
<th>Phenology</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
<th>Value 4</th>
<th>Value 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milky</td>
<td>0.155</td>
<td>0.257</td>
<td>0.257</td>
<td>0.603</td>
<td>0.546</td>
</tr>
<tr>
<td>7-8</td>
<td>-0.098</td>
<td>0.269</td>
<td>0.269</td>
<td>0.365</td>
<td>0.715</td>
</tr>
<tr>
<td>Flowering</td>
<td>-0.022</td>
<td>0.265</td>
<td>0.266</td>
<td>0.082</td>
<td>0.935</td>
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</table>

**Predation by birds**

<table>
<thead>
<tr>
<th>Term</th>
<th>Coefficient</th>
<th>SE</th>
<th>Wald</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-4.581</td>
<td>0.608</td>
<td>7.538</td>
<td>7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Habitat: Maize</td>
<td>1.708</td>
<td>0.460</td>
<td>3.716</td>
<td>1</td>
<td>0.002</td>
</tr>
<tr>
<td>Phenology: Ripe</td>
<td>0.912</td>
<td>0.279</td>
<td>3.264</td>
<td>1</td>
<td>0.001</td>
</tr>
<tr>
<td>Isolation 1</td>
<td>5.974</td>
<td>2.158</td>
<td>2.768</td>
<td>1</td>
<td>0.005</td>
</tr>
<tr>
<td>Habitat: Forest</td>
<td>-1.632</td>
<td>10.980</td>
<td>1.487</td>
<td>1</td>
<td>0.137</td>
</tr>
<tr>
<td>Isolation 4</td>
<td>-0.376</td>
<td>0.261</td>
<td>1.439</td>
<td>1</td>
<td>0.150</td>
</tr>
<tr>
<td>Phenology: Milky</td>
<td>0.398</td>
<td>0.298</td>
<td>1.335</td>
<td>1</td>
<td>0.182</td>
</tr>
<tr>
<td>Phenology: Flowering</td>
<td>-0.280</td>
<td>0.341</td>
<td>0.819</td>
<td>1</td>
<td>0.413</td>
</tr>
<tr>
<td>Phenology: Early Milky</td>
<td>-0.226</td>
<td>0.336</td>
<td>0.673</td>
<td>1</td>
<td>0.501</td>
</tr>
<tr>
<td>Phenology: BBCH17-18</td>
<td>-0.124</td>
<td>0.328</td>
<td>0.377</td>
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<td>0.706</td>
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**Predation by mammals**

<table>
<thead>
<tr>
<th>Term</th>
<th>Coefficient</th>
<th>SE</th>
<th>Wald</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-2.452</td>
<td>1.040</td>
<td>2.358</td>
<td>1</td>
<td>0.18</td>
</tr>
<tr>
<td>Habitat: Maize</td>
<td>-2.784</td>
<td>0.328</td>
<td>8.482</td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>Area</td>
<td>0.118</td>
<td>0.025</td>
<td>4.778</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Isolation 2</td>
<td>-10.410</td>
<td>2.199</td>
<td>4.732</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Habitat: Forest</td>
<td>-0.513</td>
<td>0.296</td>
<td>1.736</td>
<td>1</td>
<td>0.083</td>
</tr>
<tr>
<td>Phenology: Flowering</td>
<td>0.401</td>
<td>0.436</td>
<td>0.918</td>
<td>1</td>
<td>0.359</td>
</tr>
<tr>
<td>Phenology: Milky</td>
<td>-0.438</td>
<td>0.508</td>
<td>0.862</td>
<td>1</td>
<td>0.389</td>
</tr>
<tr>
<td>Phenology: Ripe</td>
<td>-0.079</td>
<td>0.473</td>
<td>0.167</td>
<td>1</td>
<td>0.867</td>
</tr>
<tr>
<td>Phenology: Early Milky</td>
<td>-0.070</td>
<td>0.473</td>
<td>0.148</td>
<td>1</td>
<td>0.882</td>
</tr>
<tr>
<td>Phenology: BBCH17-18</td>
<td>0.003</td>
<td>0.464</td>
<td>0.005</td>
<td>1</td>
<td>0.996</td>
</tr>
</tbody>
</table>
Article V

Predation pressure in maize across Europe and Argentina: an intercontinental comparison
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Predation pressure in maize across Europe and in Argentina: an intercontinental comparison

Marco Ferrante¹, Gábor L. Lövei¹, Serena Magagnoli², Lenka Minarcikova³, Elena Larisa Tomescu⁴, Giovanni Burgio², Ludovit Cagan³, Mihael Cristin Ichim⁴

¹Department of Agroecology, Aarhus University, Flakkebjerg Research Centre, Forårsvej 1, DK4200 Slagelse, Denmark

²Dipartimento di Scienze Agrarie, Area Entomologia, Università di Bologna, Bologna, Italy.
serena.magagnoli4@unibo.it; giovanni.burgio@unibo.it

³Department of Plant Protection, Faculty of Agrobiology and Food Resources, Slovak Agricultural University, Tr. A. Hlinku 2, SK-949 76 Nitra, Slovakia;
minarcik.lenka@gmail.com, ludovit.cagan@gmail.com

⁴NIRDBS/”Stejarul” Research Centre for Biological Sciences, 6 Alexandru cel Bun St., Piatra Neamt, 610004, Romania. lt.tomescu@yahoo.com; cichim@hotmail.com

Running title: Predation on artificial caterpillars in maize

Corresponding author: Marco Ferrante, Department of Agroecology, Aarhus University, Flakkebjerg Research Centre, Forsøgsvej 1, DK4200 Slagelse, Denmark. E-mail: marco.ferrante@agro.au.dk
Abstract

Humankind draws important benefits from large-scale ecological processes, termed ecosystem services. They should continue functioning unharmed, yet the status of several of them is declining. Therefore, reliable monitoring methods are essential for tracking the status of ecosystem services. Predation is the ecological process behind the ecosystem service of natural pest control. We used green plasticine caterpillars to monitor predation pressure, and to obtain baseline data on predator activity in transgenic Bt vs. non-Bt maize fields in Old and New World countries. Predation pressure was measured at ground and canopy levels using an identical, small-plot experimental design in Denmark, Slovakia, Romania, Italy and Argentina. Total predation rate in maize was 11.7%d$^{-1}$ (min. 7.2%d$^{-1}$ in Argentina, max. 29%d$^{-1}$ in Romania). Artificial caterpillars were attacked both by invertebrates (mostly chewing insects with 42.0% of the attack marks, and ants with 7.1%, but also predatory and parasitoid wasps, spiders and slugs), and vertebrates (small mammals, 25.5%, and birds 20.2%). Total predation at ground level (15.7%d$^{-1}$) was significantly higher than in maize canopies (6.0%d$^{-1}$) in all countries, except Argentina. We found no significant differences between predator pressure in Bt vs. non-Bt maize plots. The artificial caterpillar method provided comparable, quantitative data on predation intensity, and proved to be suitable for monitoring natural pest control. This method usefully expands the existing toolkit by directly measuring ecological function rather than structure.

Keywords: artificial caterpillars, ecosystem services, mortality, sentinel prey, transgenic plants, AMIGA Project
Introduction

Ecosystem services (ESs) are outcomes of ecological processes that provide essential benefits to humans (Daily, 1997). ESs are classified as provisioning, regulating and supporting, and cultural services (Maes et al., 2016). The nature of the relationship between ESs and biodiversity has been long debated (Peterson et al., 1998, Tscharntke et al., 2005), but there is general consensus that high biodiversity enhances ESs (Isbell et al., 2011, Harrison et al., 2014). Because of several unfavourable aspects of human activities (Ceballos et al., 2015), the status of many ESs is not satisfactory (MEA, 2005, Carpenter et al., 2009). This raises the need for monitoring ESs (Meyer et al., 2015).

Genetically modified (GM) transgenic plants have been commercially grown since 1995, and have generated profound changes in agricultural practices, leading to large, often unexpected environmental consequences (Wu et al., 2008, Lu et al., 2010). The general need for environmental impact assessment, including ESs was raised early (Lövei, 2001), and the effect on ESs is now a major concern in Europe in numerous fields (Maltby et al., 2017). In the European Community, post-market environmental monitoring (PMEM) of GM crops is also mandatory (Directive 2001/18/EC) for a possible early detection of unwanted, unexpected impacts (EFSA 2011), but detailed guidance about how such monitoring should be conducted, and how empirical data should be collected, is lacking: The EFSA Guidance (EFSA 2011) relies on farmer questionnaires, and scanning the literature for outputs of individual research projects. Between 2012-2016, 15 European countries and Argentina collaborated within the AMIGA project ("Assessing and Monitoring the Impacts of Genetically Modified Plants on Agro-ecosystems") which aimed to develop standardised protocols and monitoring tools for PMEM (Arpaia et al., 2014).
Historically, monitoring ESs was done by following changes in the diversity and abundance of the species providing the services (“service providing units”, SPUs) (Kontogianni et al., 2010). This creates a taxonomic impediment, because identifying special invertebrate groups requires specialist, or at least parataxonomist training. Additionally, the soundness of this approach is doubtful, and measuring directly ESs should be preferred (Meyer et al., 2015). In the case of biological control provided by natural enemies, which is an important ES among the regulating and supporting ones (Maes et al., 2016), the use of natural enemy diversity or abundance as indicator may easily result in fallacious conclusions. It is not straightforward, for example, that increasing predator diversity enhances the control of pest populations, because negative interactions among insect predators, such as cannibalism or intraguild predation, are very frequent (Rosenheim et al., 1995).

Insect predators are important in many pest control strategies (Begg et al., 2016, Gurr et al., 2017), and tracking their activity is desirable under environmental monitoring. Extensive literature exists on methods how to detect arthropod predation (Kidd & Jervis, 2005, Furlong, 2015, Birkhofer et al., 2017), but only the sentinel prey method allows obtaining quantitative estimates, and controlling most experimental variables (Meyer et al., 2015). In particular, artificial caterpillars made of plasticine (Howe et al., 2009) are cheap, easy to use, and can potentially give more comparable results compared to other sentinel prey (Lövei & Ferrante, 2017).

In a transcontinental experiment, we used artificial caterpillars placed at ground and canopy levels to record predation intensity in maize, one of the most important crops worldwide (Ranum et al., 2014), in five partner countries within the AMIGA project: Argentina, Denmark, Italy, Romania, and Slovakia. In part, the aim was to establish a baseline for later
comparisons and monitoring, and in part, we also compared predation pressure in small transgenic GM vs. non-GM maize plots.

We hypothesised that (H1) predation pressure would be higher on the ground than in the maize canopy. We expected this because of the high diversity of ground-active natural enemies in European agricultural fields, which include several predatory arthropod groups, including ground beetles (Coleoptera: Carabidae, Lövei & Sunderland, 1996), spiders (Sunderland & Samu, 2000), ants, and rove beetles (Dahl, 2013), among others. Further, we hypothesized (H2) that predation pressure in Argentina will be higher than in Europe, because maize relatives (Zea spp.) are native to the Americas (Ranum et al., 2014), where there has been an evolutionary opportunity for a rich trophic network associated with that plant to develop, while maize was only brought to Europe in 1492 (Mann, 2011), thus have not had this evolutionary opportunity. We also expected that predation pressure will reflect the biodiversity gradient within Europe from north-west to south-east: the natural enemy assemblages in cultivated land in general are much more species-rich in SW Europe than further north and west (Mészáros et al., 1984, Báldi et al., 2013), although agricultural practice and pesticide use can have important influence (Lüscher et al., 2014). Agriculture in Europe is more intensive and relies on higher chemical inputs in the northern and north-western countries, with deleterious effects on biodiversity (De Ponti et al., 2012). Consequently, (H3) we expected that predation intensity in Northern Europe would be lower than in Southern, and especially south-eastern Europe, as biodiversity generally increases towards the Equator (Gaston, 2000). Both factors would generate higher predation pressure in SE than NW Europe.
Our results indicated high predation pressure at or near ground level, but much lower on maize canopies. There was higher predation on artificial caterpillars on maize canopies in Argentina vs. Europe, and our hypothesis was supported about the predation pressure gradient within Europe: significantly more caterpillars were attacked in Slovakia and Romania than in Denmark or Italy. The use of artificial caterpillars is eminently suitable as a monitoring method in post-release monitoring of field-grown transgenic crops.
Methods

Study sites

Predation pressure was measured using the artificial caterpillar method (Howe et al., 2009) near Slagelse, on the island of Zealand in Denmark (2014-2016), near Nitra in western Slovakia (2013-2014), near Roman in north-east Romania, and in Emilia-Romagna in Northern Italy (both 2014-2015), and near Córdoba in central Argentina (2016) (for precise locations, see Table S1). The same field arrangement was used in all countries, except in Italy where growing transgenic plants outdoors is not allowed. The experimental design consisted of twenty randomised plots; ten of transgenic maize, and ten of its near-isogenic line. In Romania, an additional experiment using three treatments (transgenic maize, isogenic line grown conventionally, and isogenic line grown using an Integrated Pest Management method, four plots for each treatment) was also performed, and data are included from this additional experiment. In Denmark, Slovakia and Romania, identical maize cultivars were used, but not in Argentina, where the local plant varieties were used (for details see Table S1).

Measuring predation rates

Individual caterpillars (15mm length x 3mm diameter in Denmark, Romania, and Argentina, and 20mm length x 3mm diameter in Slovakia, and Italy) were glued to a piece of reed (ca.35 mm long, 6 mm wide) and placed at ground level touching maize stems as if the caterpillars were climbing the stem (Fig. 1). In each plot, sentinels were placed at the base of eight randomly selected plants (only 4 in Romania), but with the constraint that they could not be any of the two neighbouring plants in any direction, nor the outmost plant of the plot. A
further eight larvae were pinned (Denmark from 2014 onwards, and Argentina) or glued (Denmark in 2013, Slovakia, and Italy) on the leaves of the same plants or the one nearby on the third leaf from the top (starting when plants were approximately 15-20 cm tall). Predation intensity in the maize canopy was not measured in Romania.

In 2013, five sentinel prey sessions were performed with a total of 1280 caterpillars in Denmark, and three sessions with 960 caterpillars in Slovakia; in 2014, four session with 1120 and 512 caterpillars in Denmark and Romania, respectively, three sessions with 960 caterpillars in Slovakia, and five sessions with 2144 caterpillars in Italy. In 2015, five sessions were done with 1120 exposed caterpillars in Denmark, and three each with 384 and 1056 caterpillars in Romania, and Italy, respectively. In Argentina, during the southern summer of 2015-2016, 1601 caterpillars were exposed to predators during seven sessions. This led to an overall total of 11,137 artificial caterpillars used.

In all sessions, artificial caterpillars were exposed for 24h, after which they were collected and eventual predation marks identified using a hand-held lens (12x magnification) in the field, and cross-checked in the laboratory under a binocular microscope using our own photographic records or published information (Ferrante et al., 2014, Low et al., 2014). All identifications were done by either MF or GLL except the Italian samples, which were identified by SM. Multiple attack marks by the same predator group were assumed to originate from the same predator, but signs by different predator types were considered independent attacks. Very few caterpillars (294/11,456, 0.02%) were lost, and these were excluded from the analysis. Most of these (n=110) were artificial caterpillars glued to leaves.
in Denmark in 2013, which were dislodged by neighbouring leaves moved by the wind. These were all left out even if they were found on the ground at inspection.

**Statistical analysis**

Sentinels on the ground and in the maize canopy were on the same plant. It is unlikely that they were not independent, as very few ground-active predators will climb maize plants (Lövei & Szentkirályi, 1984), but we analysed them separately. Predation on artificial caterpillars was analysed by generalised linear mixed models (GLMM). Maize type (GM or non-GM), and country were fixed factors, plot was a random factor, and there were no interactions between factors. The post-hoc Tukey $t$-test was used to determine the significance of the results. Mean predation rates within the same country in different years were tested using the post-hoc Tukey test on a logistic regression with country and year as fixed factors. Similarity among predator assemblages was calculated using the Bray-Curtis dissimilarity index. All statistical analyses were performed using the statistical program R, version 3.1.1 (R Core Team, 2014), and the ‘vegan’ package (Oksanen et al., 2015).
Results

Totally, 11.7% (1265/10,843) of the artificial caterpillars were attacked after the 24h exposure. The identified predator groups included invertebrates (mostly chewing insects with 42.0% of the bites, ants with 7.1%, predatory and parasitoid wasps, slugs, spiders and unknown arthropods, with 1.9% all together), and vertebrates (small mammals, 25.5%, and birds 20.2%). Total predation was significantly higher (Student’s $t$-test: $t = 16.8$, $P < 0.001$) at ground level (mean = 15.7%d$^{-1}$, SD = 36.3%d$^{-1}$, $N = 6379$) than on the maize canopy (mean = 6.0%d$^{-1}$, SD = 23.7%d$^{-1}$, $N = 4464$). There was no significant difference (GLMM: $Z = 0.7$, $P > 0.05$) between overall predation rates in plots of transgenic Bt- (mean = 12.7%d$^{-1}$, SD = 33.3%d$^{-1}$, $n = 3773$) vs. non-Bt maize (mean = 11.1%d$^{-1}$, SD = 31.4%d$^{-1}$, $n = 7070$).

Predation by country

Predation intensity on caterpillars at ground level (Fig 2) at the Romanian site was significantly higher (Tukey’s $t$-test, $z = -3.4 - 10.1$, $P < 0.007 - 0.001$) than in any other studied location. Ground-placed caterpillars in Slovakia were also attacked significantly more than in Denmark, Italy, or Argentina (Tukey’s $t$-test, $z = 4.0 - 7.5$, $P < 0.001$ for all comparisons). There were no significant differences (Tukey’s $t$-test, $z = 0.2$, $P = 1.0$) in ground-level predation intensity between Italy and Denmark, but both were significantly higher (Tukey’s $t$-test, $z = 3.5- 3.9$, $P = 0.001 - 0.004$) than in Argentina.

Predation intensity on maize-canopy in Slovakia was significantly higher (Tukey’s $t$-test, $z = 2.9 - 8.0$, $P < 0.001$, Argentina $P = 0.014$) than elsewhere (Fig 2). Maize canopy predation intensities were not significantly different (Tukey’s $t$-test, $z = -1.4$, $P = 0.493$) between
Denmark and Argentina, but both were significantly higher than the canopy predation measured in Italy (Table 1).

**Predator assemblages**

In Romania, caterpillars were only placed at ground level and most attacks were by vertebrates: small mammals were responsible for 68.1% of the bites, birds for 17.6%, and the share of unidentified vertebrates was 3.7%. Invertebrates included chewing insects (11.4% of attacks), and ants (0.8%). In Slovakia, most attacks were by invertebrates (chewing insects 39.0%, ants 24.0%). A few spider (1.3%), slug and parasitoid marks (0.6% for each) were also found. Attacks by vertebrates were fewer: small mammals had a 31.0% share, and birds were responsible for 8.3% of the attacks. In Denmark, more attacks were by vertebrates (birds, 43.5%, small mammals, 11.1%), than invertebrates (chewing insects, 29.7%, plus two spiders, and a single wasp attack mark). Unknown bites accounted for 15.3% of the bites. In Italy, 74.2% of the attacks were by invertebrates (chewing insects 66.9%, wasps 3.1%, ants 2.3%, unidentified arthropods 1.9%); vertebrates made 24.2% of the attacks (birds 15.4%, small mammals 8.8%). Almost all predators in Argentina were chewing insects (93.9% of the bites); only a few marks were attributed to ants (5.3%), and a single caterpillar (0.9%) had a bird mark.

We also compared the activity profiles of the predator assemblages. Argentina and Italy had similar predator assemblage activity profiles both at ground and maize-canopy levels (Figs S1, S2). In the case of ground-level predation, assemblage activity profiles in Romania and
Slovakia were similar, and the Danish one close but separate from this cluster. At canopy level, Denmark and Slovakia demonstrated similar profiles (Table 2).

**Predation within country, between years**

Multi-year data were available from Romania, Slovakia, Denmark, and Italy (Table S1). In Romania, predation rate in 2014 at ground level (mean = 37.1%d⁻¹, SD = 48.4%d⁻¹, n = 304) was significantly higher (Tukey’s t-test, z = -5.0, P < 0.001) than in 2015 (mean = 17.1%d⁻¹, SD = 37.7%d⁻¹, n = 228).

The opposite time trend was found in Slovakia: predation rate on ground caterpillars in 2013 (mean = 14.8%d⁻¹, SD = 35.5%d⁻¹, n = 480) was significantly lower (Tukey’s t-test, z = 5.0, P < 0.001) than in 2014 (mean = 28.1%d⁻¹, SD = 45.0%d⁻¹, n = 477). Predation rate in the maize canopy in 2013 (mean = 12.7%d⁻¹, SD = 33.3%d⁻¹, n = 480) was higher, even if not significantly (Tukey’s t-test, z = -1.4, P = 0.841) than in 2014 (mean = 9.8%d⁻¹, SD = 29.8%d⁻¹, n = 479).

In Denmark, there was no significant difference (Tukey’s t-test, z = -0.5, P = 1.0) between predation rates in 2013 (mean = 17.0%d⁻¹, SD = 37.6%d⁻¹, n = 787) and 2014 (mean = 16.1%d⁻¹, SD = 36.7%d⁻¹, n = 635) but it decreased significantly (Tukey’s t-test, z = -6.5- -7.9 both P < 0.001) in 2015 (mean = 4.4%d⁻¹, SD = 20.5%d⁻¹, n = 639). Similarly, predation rates in the maize canopy in 2013 (mean = 3.2%d⁻¹, SD = 17.7%d⁻¹, n = 371) and 2014 (mean = 4.3%d⁻¹, SD = 20.3%d⁻¹, n = 466) were not significantly different (Tukey’s t-test, z = 0.7, P = 0.993), but in 2015 (mean = 9.0%d⁻¹, SD = 28.6%d⁻¹, n = 412), predation was significantly
higher (Tukey’s t-test, $z = 3.2$, $P = 0.02$) than 2013, and marginally so (Tukey’s t-test, $z = 2.8$, $P = 0.1$) than in 2014.

In Italy, predation rate at ground level in 2014 (mean = $11.5\%d^{-1}$, SD = $31.9\%d^{-1}$, $n = 1195$) was significantly lower (Tukey’s t-test, $z = 3.2$, $P = 0.055$) than in 2015 (mean = $17.1\%d^{-1}$, SD = $37.7\%d^{-1}$, $n = 527$), but there was no significant difference (Tukey’s t-test, $z = 0.1$, $P = 1.0$) between predation rates at maize canopy level in 2014 (mean = $2.2\%d^{-1}$, SD = $14.8\%d^{-1}$, $n = 938$) and 2015 (mean = $2.3\%d^{-1}$, SD = $15.0\%d^{-1}$, $n = 524$).

**IPM experiment in Romania**

In Romania, there were no significant differences (Tukey’s t-test: $Z = -1.3$-0.3, all $P > 0.05$) between predation rates in Bt maize plots (mean = $34.6\%d^{-1}$, SD = $47.8\%d^{-1}$, $n = 104$), non-Bt plots grown conventionally (mean = $31.4\%d^{-1}$, SD = $46.6\%d^{-1}$, $n = 102$), or IPM methods (mean = $23.4\%d^{-1}$, SD = $42.5\%d^{-1}$, $n = 107$).
Discussion

Our detected predation rates were in the high range with respect to available data from various geographical locations and habitats (Lövei & Ferrante, 2017). Data, however, are scarce from agricultural habitats, and in some of these (e.g. Lemessa et al., 2015), methodological differences make comparison not possible. Our data on predation rates are conservative, because they do not include predation pressure by predators that use traps to capture active prey. For example, web-building spiders (Riechert & Lockley, 1984) and parasitoids (Kidd & Jervis, 2005) are important natural enemies, yet their impact is certainly underrepresented by our method. Differences in the composition of the predator assemblage only partially can explain the registered differences. The north-south gradient was only partially apparent in our studies, in that Italy did not have the highest predation pressure, which can be related either to the peninsular position, leading to a lower-than-expected biodiversity, or a practice of chemical-intensive agriculture, typical of much of Western and Southwestern Europe (Kleijn et al., 2009). The effect of agricultural practice is also seen in the higher predation pressure in Eastern and South-eastern Europe, where agriculture is still less intensive, resulting in higher diversity and more natural pest control (Kleijn et al., 2009). Curiously, predation pressure in Argentina was not higher than in Europe overall, but predation pressure in the maize canopy was equal to the ground-level one, being the only such among the locations examined. This is consistent with the long evolutionary history hypothesis, at least for above-ground (canopy) predators.

Lövei and Ferrante (2017) analysed the predation intensity on artificial caterpillars in forests by geographical latitude, and found no significant increase from north to south. At the time, there were insufficient data to attempt a similar analysis for cultivated habitats. With several
new data points added by this paper, such an analysis can now be attempted (Fig. 3). Interestingly, there was a significant positive relationship found for predation by vertebrates, but not for invertebrates (Fig. 3).

Our hypothesis of higher predation pressure at ground level vs. on maize canopies in Europe was supported. Although a high number of species could be found in maize in Europe (Mészáros, 1984, Dahl, 2013), many of these are probably “tourists” that have no proven trophic link to maize. In Europe, there are few specific herbivores of maize, although several Lepidoptera and aphids have included this relatively new plant in their host plant range (Dahl, 2013). A curious example is the pyralid *Ostrinia nubilalis* that was inadvertently transported to North America, where it became an important pest, giving the original impetus to develop the insect resistant transgenic (Bt) maize (Koziel et al., 1993). Overall, however, the soil-based food web is less maize-specific, and due to a longer evolution under European conditions, it is not surprising that the European ground-active predators colonised the maize–dominated habitats successfully.

We found no difference in overall predation pressure between our GM vs. non-GM maize plots at any location. This is in line with no aversion of *Bt* plants by egg-laying insects (Obonyo et al., 2008). *Bt*-residues can be detected in the soil (Icoz & Stotzky, 2008) and in soil-based food chains (Andow & Zwahlen, 2016) but their eventual effect is unclear. Earthworms do not seem to be adversely affected by *Bt* maize (Zeilinger et al., 2010), and they are important prey for ground beetles (Lövei & Sunderland, 1996). Ground beetles are a very common arthropod group in maize fields (Lövei, 1984, Lee & Albajes, 2016), and may be responsible for a substantial share of attacks on artificial caterpillars (Mansion-Vaquié et
al., 2017). A very high number of carabids captured in the experimental plots in Denmark (Di Grumo & L Lövei, 2016) indicates that there was dispersal of ground-active arthropods between our small plots (10mx10m, with 5 m separation) which would confound possible differences.

Monitoring has become a very important goal, mainly due to the multitude of harms we are causing nature, as well as to processes useful to ourselves, humans (Carpenter et al., 2009). In many situations, including the field-grown transgenic crops, the topic our AMIGA Project was tasked to work on, monitoring is obligatory (Arpaia et al., 2014). In the case of the PMEM requirement, recommendations and instructions were lacking. In general, however, the practice of monitoring leaves much to desire. All too often the basic steps of identifying the necessary elements, the indicandum (the phenomenon of interest that we want information on), the appropriate indicator (the organism that can indicate the phenomenon with the desired precision and sensitivity), and the index (the appropriate parameter to measure), are lacking (Lövei 2014). The existence of the universal principle of indication states that every organism is an indicator. By its very presence, it indicates the presence of a set of conditions that are necessary for its existence and survival at the given location. This is great relief to specialists who routinely argue that their favourite group is “a good indicator” (see, for carabids, Koivula, 2011, for syrphids, Sommaggio, 1999, for isopods, Dallinger et al., 1992, etc.).

Added to this is the “structure for function” paradox. In many cases, we are interested in the conditions, or intensity of an ecological function, mostly because of its usefulness to us. This utilitarian interest leads to the need of monitoring the status of various ecosystem services.
Due to the existing ecological toolkit, this interest in a function necessarily leads to monitoring the changes in the distribution, diversity or density of selected SPUs. Only recently have we started to realise that there is no direct nor easy translation between ecological structure and ecological function, which lead to calls to develop and use methods by which we can track function intensity directly (Meyer et al., 2015), rather than their proxies in terms of SPUs.

The AMIGA Project articulated the need for using both, and our paper is the summary of the field experiments aiming to accumulate empirical evidence of the features, practicability and ease of use of the artificial sentinel prey method. This was successfully used in various countries in Europe and easily adapted to South American conditions, providing useful information for monitoring, but also to detect the effect of native vegetation fragments on predation pressure in maize fields (Ferrante et al. submitted). The cryptically coloured artificial caterpillar prey proved to be attractive to a wide range of potential predators, and proved easy to use under field conditions. Some biological knowledge, allowing the recognition of marks left by various predators, increases the information to be obtained from the method, but a rough estimate of general predation pressure can be done with minimum of such knowledge. We conclude that the artificial caterpillar method is useful to monitor the intensity of natural pest control under a wide range of field conditions, and is useful for the large-scale environmental monitoring of field-grown transgenic plants as mandatory in the European Community.
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Disclosure

The authors disclose no conflicts of interest of any kind associated with this manuscript.
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californiae* Swinhoe (Lepidoptera: Crambidae) and *Sesamia calamistis* Hampson

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Text of the figures

**Fig. 1** An artificial caterpillar exposed at the base of a maize plant, as if it would be climbing the maize stem. Photo: S. Magnanoli.

**Fig. 2** Predation intensity by country and predator group, at ground level (circles) and on canopy-placed (triangles) artificial caterpillars in maize fields in four European countries and Argentina.

**Fig. 3** The relationship between geographical latitude (expressed in degrees) and predation rates measured using artificial caterpillars by invertebrate and vertebrate predators in cultivated areas. The solid lines represent the fit of a linear regression, dashed lines indicate ± 95% CI. Regression equations: (invertebrates) $y = 3.55702 + 0.03987x$, $P = 0.278$, adj. $R^2 = 0.01229$; (vertebrates) $y = 1.56712 + 0.11638x$, $P = 0.057$, adj. $R^2 = 0.1113$. Circles indicate published data, while crosses mark new data from this article.
Table 1. Predation on artificial caterpillars in maize fields at ground and canopy levels for each country partner in the AMIGA project. Data are means ± SD. Sample sizes are in parentheses.

<table>
<thead>
<tr>
<th>Country</th>
<th>Predation rate (%) on caterpillars placed on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ground</td>
</tr>
<tr>
<td>Denmark</td>
<td>12.8 ± 33.4 (2061)c</td>
</tr>
<tr>
<td>Slovakia</td>
<td>21.4 ± 41.0 (957)b</td>
</tr>
<tr>
<td>Romania</td>
<td>29.0 ± 45.4 (845)a</td>
</tr>
<tr>
<td>Italy</td>
<td>13.2 ± 33.8 (1722)c</td>
</tr>
<tr>
<td>Argentina</td>
<td>7.3 ± 26.0 (794)d</td>
</tr>
</tbody>
</table>

Tukey’s t-test was used to test for significance between countries and within the same caterpillar position.
Table 2. Relative predation rates (% of all predation events) by different predatory groups in maize fields at ground and canopy levels in each country partner in the AMIGA project, organised by geographical latitude.

<table>
<thead>
<tr>
<th>Prey position, country</th>
<th>Insects (except ants)</th>
<th>Ants</th>
<th>Birds</th>
<th>Mammals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ground level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denmark</td>
<td>29.2</td>
<td>0</td>
<td>45.5</td>
<td>14</td>
</tr>
<tr>
<td>Slovakia</td>
<td>37.1</td>
<td>15.1</td>
<td>9.8</td>
<td>44.9</td>
</tr>
<tr>
<td>Romania</td>
<td>11.4</td>
<td>0.8</td>
<td>17.6</td>
<td>68.2</td>
</tr>
<tr>
<td>Italy</td>
<td>72.3</td>
<td>3.1</td>
<td>14.1</td>
<td>10.1</td>
</tr>
<tr>
<td>Argentina</td>
<td>87.9</td>
<td>10.3</td>
<td>1.7</td>
<td>0</td>
</tr>
<tr>
<td><strong>Maize canopy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denmark</td>
<td>33.3</td>
<td>0</td>
<td>36.2</td>
<td>0</td>
</tr>
<tr>
<td>Slovakia</td>
<td>44.4</td>
<td>40.7</td>
<td>5.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Romania</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Italy</td>
<td>72.7</td>
<td>0</td>
<td>24.2</td>
<td>0</td>
</tr>
<tr>
<td>Argentina</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Fig. 1 An artificial caterpillar exposed at the base of a maize plant, as if it would be climbing the maize stem.
Photo: S. Magnanoli.

136x205mm (220 x 220 DPI)
Fig 2 Predation intensity by country and predator group, at ground level (circles) and on canopy-placed (triangles) artificial caterpillars in maize fields in four European countries and Argentina.

159x95mm (220 x 220 DPI)
Fig 3 The relationship between geographical latitude (expressed in degrees) and predation rates measured using artificial caterpillars by invertebrate and vertebrate predators in cultivated areas. The solid lines represent the fit of a linear regression, dashed lines indicate ± 95% CI. Regression equations:
(invertebrates) $y = 3.55702 + 0.03987x$, $P = 0.278$, adj. $R^2 = 0.01229$; (vertebrates) $y = 1.56712 + 0.11638x$, $P = 0.057$, adj. $R^2 = 0.1113$. Circles indicate published data, while crosses mark new data from this article.
Supplementary material

Fig. S1 Predator assemblage similarity for ground-level predation in maize in partners countries within the AMIGA project.
Fig. S2. Predator assemblage similarity for maize canopy-level predation in maize in partner countries within the AMIGA project.
Table S1. Details of location, plot sizes and cultivation of the experimental maize fields of each country where predation intensity was measured using artificial caterpillars. Data are organised by the starting year.

<table>
<thead>
<tr>
<th>Country, location</th>
<th>Coordinates</th>
<th>Year</th>
<th>Maize cultivars and treatments</th>
<th>Plot size</th>
<th>Sowing and harvesting date</th>
<th>Chemical inputs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flakkebjerg Research Centre, Aarhus</td>
<td>55°19'19&quot;N 11°23'25&quot;E</td>
<td>2013-2015</td>
<td>DKC3872YG; DKC3871 (control)</td>
<td>10m x 9m, separation 5m bare ground</td>
<td>27 May-6 Nov 2013, 12 Apr–10 Oct 2014, 28 Apr–15 Oct 2015</td>
<td>2 herbicide treatments; 1 &amp; 4 wks after planting</td>
</tr>
<tr>
<td>University</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slovakia</td>
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</tr>
<tr>
<td>Borovce</td>
<td>48°34'83&quot;N 17°43'30&quot;E</td>
<td>2013-2014</td>
<td>DKC3872YG; DKC3871 (control)</td>
<td>10m x 10m, separation 5m barley, cut short</td>
<td>9 May-24 Oct 2013, 28 Apr–6 Nov 2014</td>
<td>2014: 1 herbicide treatment (Maister 0.15lha⁻¹ + Istroekol 2.0lha⁻¹) 3 wk after planting, 2015: 2 herbicide treatments (Dual Gold 1.25lha⁻¹ + Mustang 0.8lha⁻¹); 1 &amp; 4 wks after planting</td>
</tr>
<tr>
<td>Romania</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secuieni Agricultural Research &amp;</td>
<td>46°51'05&quot;N 26°51'33&quot;E</td>
<td>2014-2015</td>
<td>DKC3872YG; DKC3871 (control)</td>
<td>10m x 10m, separation 5m short grass</td>
<td>7 May-14 Oct 2014, 8 May-19 Oct 2015</td>
<td>2 herbicide treatments (Buctril Universal0.8lha⁻¹+Equip 1.75lha⁻¹); 4 &amp; 8 wks after planting</td>
</tr>
<tr>
<td>Development Station</td>
<td></td>
<td></td>
<td>DKC3872YG; DKC3871 conventional &amp; IPM</td>
<td>30m x 30m, separation 5m short grass</td>
<td>7 May-14 Oct 2014, 8 May-19 Oct 2015</td>
<td>4 isogenic plots 2 insecticide-treatments(Avaunt150 SC 0.25lha⁻¹); 6 &amp; 8 wks after planting</td>
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<td></td>
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<td></td>
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<tr>
<td>Italy</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>San Pietro in Casale</td>
<td>44°40'54&quot;N 11°25'35&quot;E</td>
<td>2014</td>
<td>DKC7677</td>
<td>1 field 0.5ha</td>
<td>24 Mar - wk2 Aug 2014</td>
<td>pesticide(0.5% Teflurin,15kgha⁻¹), at planting + pre-emergency herbicide (terbuthylazine + sulcotrione +</td>
</tr>
<tr>
<td>Location</td>
<td>Latitude/Longitude</td>
<td>Year</td>
<td>Variety</td>
<td>Size (ha)</td>
<td>Start - End Period</td>
<td>Treatment Details</td>
</tr>
<tr>
<td>------------------------</td>
<td>---------------------</td>
<td>-----------</td>
<td>------------------</td>
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<td>--------------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Cadriano</td>
<td>44°33'18&quot;N 11°24'51&quot;E</td>
<td>2014</td>
<td>AGN 583</td>
<td>1 field</td>
<td>0.5ha</td>
<td>pesticide (0.4% lambda-cyhalothrine 10 kg ha(^{-1})), at planting + pre-emergency herbicide (28.9% S-Metolachlor + 17.4% Terbutilazin); date unknown</td>
</tr>
<tr>
<td>Cadriano</td>
<td>44°33'01&quot;N 11°24'38&quot;E</td>
<td>2014</td>
<td>AGN 583</td>
<td>2 fields</td>
<td>0.3ha each</td>
<td>pesticide (0.4% lambda-cyhalothrine 10 kg ha(^{-1})), at planting + pre-emergency herbicide (28.9% S-Metolachlor + 17.4% Terbutilazin); date unknown</td>
</tr>
<tr>
<td>Cadriano</td>
<td>44°32'54&quot;N 11°24'55&quot;E</td>
<td>2015</td>
<td>AGN 583</td>
<td>2 fields</td>
<td>0.3ha each</td>
<td>pesticide (0.4% lambda-cyhalothrine 10 kg ha(^{-1})), at planting + pre-emergency herbicide (28.9% S-Metolachlor + 17.4% Terbutilazin); date unknown</td>
</tr>
<tr>
<td>Cadriano</td>
<td>44°32'59&quot;N 11°24'39&quot;E</td>
<td>2015</td>
<td>AGN 583</td>
<td>3 fields</td>
<td>0.17ha each</td>
<td>pesticide (0.4% lambda-cyhalothrine 10 kg ha(^{-1})), at planting + pre-emergency herbicide (28.9% S-Metolachlor + 17.4% Terbutilazin); date unknown</td>
</tr>
<tr>
<td>Argentina</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 herbicide treatment (glyphosate 2 l ha(^{-1}) + atrazin 1 kg ha(^{-1}) + 2,4-Dichlorophenoxyaceticacid 450 g ha(^{-1})); 4 wks after planting</td>
</tr>
</tbody>
</table>

* southern growing season (Sep-Jun)
Article VI

Ferrante M, Barone G, Kiss M, Bozóné-Borbáth E, Lövei GL
Predation on artificial caterpillars indicates no enemy-free time for lepidopteran larvae
Under review in
Entomologia Experimentalis et Applicata, EEA-2017-0081
Predation on artificial caterpillars indicates no enemy-free time for lepidopteran larvae

Marco Ferrante\textsuperscript{1*}, Giulio Barone\textsuperscript{1,2}, Melinda Kiss\textsuperscript{1,3}, Erna Bozóné-Borbáth\textsuperscript{1,4}, & Gabor L. Lövei\textsuperscript{1}

\textsuperscript{1} Department of Agroecology, Aarhus University, Flakkebjerg Research Centre, Forgøsvej 1, DK4200 Slagelse, Denmark

\textsuperscript{2} University of Palermo, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, 16 Viale delle Scienze, I-90128, Palermo, Italy

\textsuperscript{3} University of Agricultural Sciences and Veterinary Medicine, Faculty of Horticulture, 3-5/Manastur, Cluj-Napoca, Romania

\textsuperscript{4} 10 Battonyai street, H-5744 Kevermes, Hungary

Running title: Day vs. night predation on plasticine caterpillars

Key words: sentinel prey, aposematic coloration, defensive colouration, temperate forest, ecosystem service, foraging behaviour, mortality

*Corresponding author: Marco Ferrante, Department of Agroecology, Aarhus University, Flakkebjerg Research Centre, Forgøsvej 1, DK4200 Slagelse, Denmark. E-mail: marco.ferrante@agro.au.dk
Abstract

Herbivorous insects display various predator avoidance strategies, including chemical defences often paired with honest signals of their unpalatability, camouflage, and/or night-time activity that, by providing enemy-free time, may reduce their risk of being predated. We compared predation pressure on differently coloured artificial caterpillars, mimicking cryptic vs. aposematic coloration, during day or night in forest fragments in a cultivated landscape in Denmark. The most important predators were chewing insects (73.4% of all attacks) and small mammals (19.0%). Attacks were significantly more frequent during night- (mean=30.9%d^-1) than daytime (mean=17.0%d^-1), both for invertebrate (23.3%d^-1 vs. 12.4%d^-1) and vertebrate (8.5%d^-1 vs. 3.3%d^-1) predators. Aposematic (red) coloration did not provide protection: attack rates on red caterpillars were 30.0%d^-1 vs. 19.5%d^-1 on cryptic (green) ones. Overall, these data do not support the idea that night-time activity can provide enemy-free time for solitary, free-living caterpillars in temperate forests.
Introduction

Quantitative estimates suggest that under natural conditions, predation on caterpillars can reach extremely high levels (Lövei & Ferrante, 2017; Mira & Bernays, 2002). In order to protect themselves from predators, caterpillars evolved a great variability of defences (Greeney et al., 2012). These defences include “meaningful” colouration (Skelhorn et al., 2010), such as aposematism (communicating to potential predators toxicity/unpalatability), or camouflage (matching the background to reduce detection). Even if these colourations are considered alternative strategies to defend from visually oriented predators (Poulton, 1890), they require different conditions to be effective. Supposedly, aposematic colouration works better during the daytime when they are more apparent, while cryptic species should rather benefit from conditions which make them less detectable. Therefore, different foraging time for aposematic and cryptic caterpillars is expected (Heinrich, 1979). Evidence is mixed, however. Palatable caterpillars have various behavioural adaptations, including hiding, commuting to and from feeding sites, and snipping off partially eaten leaves (Heinrich, 1979) that may be interpreted as adaptations to reduce predation risk. In a tropical forest in Papua New Guinea, predation risk indeed was higher during day- than nighttime, but caterpillar densities were also higher during the day (Novotny et al., 1999). For herbivores, reacting to wound-induced chemical changes may be more important than confounding predators (Edward & Wratten, 1983). Larvae of two lepidopterans, the cryptic Pieris rapae L. and the aposematic Euphydryas phaeton (Drury) did not show differences in their feeding behaviour (Edwards & Wratten, 1983; Heinrich, 1979; Mauricio & Bowers, 1990). Quantitative data of predation rates on caterpillars possessing these
alternative strategies would contribute to understand prey behaviour, but direct comparisons are absent or rare (Carroll & Sherratt, 2013).

The artificial caterpillar method is a simple technique to quantify relative predation rates (Howe et al., 2009). One of the main advantage is that it allows identifying many predators (Lövei & Ferrante, 2017), although with varying levels of resolution (Low et al., 2014). Artificial caterpillars have been used to investigate the effectiveness of defensive colourations or patterns, such as eyespots (Hossie & Sherratt, 2012; 2013; Hossie et al., 2015), masquerade (Suzuki & Sakurai, 2015), or countershading (Rowland et al., 2008; Rowland et al., 2007) against predatory birds. However, mammals and insects such as ground beetles (Lövei & Sunderland, 1996), wasps, or praying mantises (Greeney et al., 2012) also use visual cues when looking for prey. Their reaction to such prey features is less known.

We compared daytime vs. and night-time predation in temperate forest fragments on plasticine caterpillars with cryptic (green) vs. aposematic (red) coloration. We used pairs of caterpillars of three different combinations: aposematic, cryptic, and mixed-pattern. Previous experiments showed that vertebrate and invertebrate predators are abundant in temperate forests (Remmel et al., 2011), and that predation rate on caterpillars is usually high (Ferrante et al., 2014; Mäntylä et al., 2008; Mäntylä et al., 2014). Our hypotheses were: (1) predation rate on mixed-pattern caterpillar pairs is higher than either on aposematic or cryptic colourations because it does not represents a real defensive pattern; (2) during the daytime, predation on aposematic colouration is lower than on cryptic or mixed-pattern colourations, while (3) at night there are no significant differences between predation rates on the three colourations. We did not make a specific
hypothesis on the difference between daily and night predation rates, as we assumed this
difference would be mostly determined by the ecology of the most common predatory group.

We found that invertebrate predation rate was significantly higher on aposematic prey while
vertebrate predation rate was significantly higher on cryptic ones. Both invertebrate and
vertebrate predation rates were significantly higher at night than during daytime.
Materials and methods

Study site and artificial caterpillars

Our study sites were around Flakkebjerg Research Station Experimental Farm (Aarhus University) in the western region of Zealand in Denmark. Three sites were selected, all of them temperate forest patches (0.3-0.7 ha) characterised by the presence of beech (*Fagus sylvatica*) and other, less common tree species (*Populus* sp., *Platanus* sp.), and situated at least 500m from each other. In order to quantify predation, we used artificial caterpillars 15mm long and 3mm of diameter made of green or red plasticine (Smeedi plus, Denmark), produced using a garlic press as in Howe et al. (2009). Green and red colours were chosen because they are part of the colourations of many cryptic and aposematic species, respectively. Even though not every vertebrate (Yokoyama & Radlwimmer, 2001) and invertebrate (Salcedo et al., 2003) predator can detect red colour *per se*, the strategy of aposematic species is mainly based on the colour contrast (Aronsson & Gamberale-Stille, 2009; Endler, 1991). We used uniform colouration, as previous studies found that internal contrast affect predators less than background contrast (Aronsson & Gamberale-Stille, 2009).

Experimental design

Our experimental design used a “prey package”, consisting of a pair of artificial caterpillars, placed on the ground about 5cm from each other, therefore considered non-independent (i.e. the two caterpillars of a pair were considered as a single caterpillar that we considered “predated” if at least one of them had an attack mark). Placing sentinels on the ground does not create unrealistic conditions, because caterpillars move frequently on the ground when they look for host plants (Jones, 1977), and can also drop when attacked or disturbed, with associated risk to
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be preyed (Cain, 1985). A “patch” was formed by the three possible combinations of the two
colours: green-green (GG), red-red (RR), green-red (GR), simulating respectively cryptic,
aposematic, and mixed-pattern colourations. In order to guarantee independence, the three pairs
of a patch were placed at 5m from each other in a triangular arrangement. At each site, we
established ten such patches (n=30 independent sentinels per site/sampling event), each of them
at least 10m from each other (Figure S1). Caterpillars were glued to pieces of bamboo to be
easily handled, set on the forest litter between 07:00-09:00 am, and checked directly in the field
using a magnifying glass (12 x magnification) 12h and 24h later, to obtain separate day and night
predation rates. At the first check (i.e. after 12h exposure), predated or missing sentinels were
replaced with new ones; after 24h, all caterpillars were removed. Predation marks were re-
checked in the laboratory under a stereomicroscope (Leica MS5, 6.3-40 x magnification). During
July-August 2015, we carried out four sampling events, including a pilot study using only two
sites. This lead to the use of a total of 660 artificial caterpillar pairs exposed. Note that during the
Danish summer the days are much longer than nights (L:D ~16h:8h), therefore we considered
our prey exposure periods were 12h daylight and 4h light at both ends of hours of darkness,
hereafter referred as day and night for simplicity.

Statistical analysis

Differences in total predation between day and night and between colourations were tested using
Student’s t-test on the mean predation rates. As invertebrate and vertebrate predators detect
colours differently, invertebrate and vertebrate predation rates were analysed separately using
two separate logistic regressions. The initial models were a full-model including colouration
(GG, RR, and GR), time (daytime or night-time), and the interaction between colouration and
time as fixed effects, and site as random effect. Model selection was done backwardly using AIC
(Akaike, 1998), and for both predator groups confirmed the models without interaction to be the best (Tables S1, S2). We used the post-hoc Tukey test to determine the significance of the results. Non-predatory marks by snails and slugs (n=26) were excluded from the analyses. Only two caterpillars from two different pairs went lost, so that no independent caterpillar was lost. All statistical analyses were performed using the statistical program R, version 3.1.1 (R Core Team, 2014), and the package lme4 (Bates et al., 2015).
Results

Twenty-three percent (158/660) of the artificial caterpillars showed signs of predation after 24h (Figure 1). Most marks were attributable to invertebrates (chewing insects with 73.4% of the bites, and ants, with 1.9%), and vertebrates (small mammals, 19%, birds, 5.1%), while unknown bites were few (n=8). Multiple attacks by different predators accounted for 6.3% of the bites.

Total predation was significantly higher (Student’s t-test: $t = -4.247, P < 0.001$) during the night (mean = 30.9%d$^{-0.5}$, SD = 46.3%d$^{-0.5}$, N = 330) than during daytime (mean = 17.0%d$^{-0.5}$, SD = 37.6%d$^{-0.5}$, N = 330). Aposematically coloured larvae were attacked significantly (Student’s t-test: $t = -2.553, P = 0.01$) more (mean = 30.0%d$^{-1}$, SD = 45.9%d$^{-1}$, N = 220) than cryptically coloured ones (mean = 19.5%d$^{-1}$, SD = 39.7%d$^{-1}$, N = 220), but there were no significant differences between mixed-pattern (mean = 22.3%d$^{-1}$, SD = 41.7%d$^{-1}$, N = 220) and cryptic or aposematic colourations (Student’s t-test: $t = -0.70218/-1.8475, P > 0.05$).

Invertebrate predation rates were almost always higher than vertebrate ones, with the only exception of cryptic colouration (GG) during the day (Table 1). Invertebrate predation rates were significantly higher (Tukey’s post hoc test, $Z = 3.732, P < 0.001$) during the night (mean = 23.3%d$^{-0.5}$, SD = 42.4%d$^{-0.5}$, N = 330) than at daytime (mean = 12.4%d$^{-0.5}$, SD = 33.0%d$^{-0.5}$, N = 330). Invertebrate predation on aposematic colouration (mean = 24.1%d$^{-1}$, SD = 42.9%d$^{-1}$, N = 220), was significantly higher (Tukey’s t-test: $Z = 3.550, P = 0.001$) than on cryptic one (mean = 11.4%d$^{-1}$, SD = 31.8%d$^{-1}$, N = 220), but there was no significant different between predation on mixed-pattern (mean = 24.1%d$^{-1}$, SD = 42.9%d$^{-1}$, N = 220) and the other two colour combinations (Tukey’s t-test: $Z = 1.578$ and $Z=2.064$, both $P > 0.05$).
Vertebrate predation rates were also significantly higher (Tukey’s test: $Z = 2.724$, $P = 0.024$) during the night (mean = 8.5%$d^{-0.5}$, SD = 27.9%$d^{-0.5}$, N = 330) than during daytime (mean = 3.3%$d^{-0.5}$, SD = 18.0%$d^{-0.5}$, N = 330), but there was no significant difference (Tukey’s t-test: $Z = -0.235$, $Z = -2.076$, both $P > 0.05$) among the different colourations (Figure 2).
Discussion

We found that in Danish temperate forest fragments, invertebrates rather than vertebrates were the main predators of artificial caterpillars. This is consistent with previous records which reported even higher predation rates (Ferrante et al., 2014), although the predation rate we found is higher than the median predation rate on artificial caterpillars in temperate forests (Lövei & Ferrante, 2017). Moreover, we found a considerable amount of marks by small mammals, which in temperate areas are often considered a relatively non-important source of arthropod mortality (Remmel et al., 2011). The low frequency of bird attacks may be instead a consequence of the prey arrangement, as most birds in these habitats are arboreal, and may search their prey mostly in the canopy (e.g. branches, leaves). Indeed, works testing bird predation routinely deploys artificial caterpillars at various heights above ground (e.g. Bereczki et al., 2014). However, it has to be remarked that the artificial caterpillar method is useful mainly to obtain relative predation rates (Howe et al., 2009) rather than absolute ones, and that the sentinel prey characteristics (lack of real behaviour or chemical cues) may “deceive” invertebrates and vertebrates differently.

In this experiment, total predation rate was higher during the night than the day, while the opposite was registered in Amazonia (Seifert et al., 2016), and Papua New Guinea (Novotny et al. 1999). Even though the sentinels in those experiments were on foliage, while ours were exposed at ground level, caterpillars will frequently occur on ground when moving between host plants, or after being dislodged by disturbance or predator attack (Castellanos et al. 2011).

Differences in the identity, behaviour and density of the main predators in the respective study areas could explain the registered differences in predation rates. Seifert et al. (2016) found that in Amazonia, ants (mostly Crematogaster sp.), which can be active both during the daytime and at
night (Menzi, 1987), were the most common predators. Likewise, ants were important predators in lowland Papua New Guinea, responsible for 58% of the registered attacks (Tvarkikova & Novotny 2012). The most common predators in our study were chewing insects and small mammals, which are usually more active at night than during the day. Most of the invertebrate marks we found were compatible with carabid attacks, which are mostly night-active (Lövei & Sunderland, 1996), and also attack the artificial caterpillars under laboratory conditions (Ferrante et al., submitted). While results by Seifert et al. (2016) are consistent with the hypothesis that night-time activity provides caterpillars with enemy-free time, this is not so in another tropical forest, in Papua New Guinea (Novotny et al., 1999), and in the light of our results, this does not seem so in temperate forests, either.

Our results indicated that invertebrates and vertebrates predators are attracted by different colourations. Even though colouration *per se* may have little importance, its combination with the ground vegetation background did have an effect on predators. Invertebrate predation was significantly higher on prey of supposedly aposematic colouration, while vertebrate predation was higher on the cryptic one, which is assumed not to have any chemical defences (even if the difference was not statistically significant). Our experimental design did not allow to clarify if this pattern is a consequence of detectability (the green colour is more difficult to find on a green background), or predator behaviour (the predator spotting the aposematic prey decides to avoid it). Demonstrating the explanatory mechanism behind predation rate on different colourations is challenging (Skelhorn et al., 2010), and it was beyond the focus of this study. The interaction between colouration and exposure time was not significant in either invertebrate or vertebrate predation models, suggesting that the two are independent of each other. Data from real
caterpillars support the idea that defensive colouration may be adopted even without a consequent foraging behaviour (Mauricio & Bowers, 1990).

These results confirmed that foraging behaviour cannot be explained by only considering predators (Edwards & Wratten, 1983), and other factors such as starvation, or secondary predator activity are likely to be more important than expected. Collecting quantitative estimates of predation on different defensive strategies may help to highlight their significance and explain prey behaviour.
Acknowledgements

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References


Ferrante M, Barone G & Lövei GL (submitted) The carabid *Pterostichus melanarius* uses chemical cues for opportunistic predation and saprophagy but not for finding healthy prey.


Text of the figures

Figure 1 Predation marks by different predators on green and red artificial caterpillars in Flakkebjerg (West Zealand, Denmark). Clockwise from top-left: chewing insect, bird, slug (not considered as a predator), and small mammal.

Figure 2 Predicted values (circles) and confidence intervals from the logistic regression for invertebrate (above) and vertebrate (below) predation rates in Flakkebjerg (West Zealand, Denmark).
Figure 1
Figure 2
Table 1 Mean rates of invertebrate and vertebrate predation (%d$^{-0.5}$ ± SD, N = 110 for all samples) on artificial caterpillars during daytime/night exposure in three forest patches in the area of Flakkebjerg (West Zealand, Denmark).

| Colouration   | Exposure time | Predation (%d$^{-0.5}$) by
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<tbody>
<tr>
<td></td>
<td></td>
<td>Invertebrates</td>
</tr>
<tr>
<td>Green-Green</td>
<td>Daytime</td>
<td>5.5 ± 22.8</td>
</tr>
<tr>
<td></td>
<td>Night</td>
<td>17.3 ± 38.0</td>
</tr>
<tr>
<td>Green-Red</td>
<td>Daytime</td>
<td>12.7 ± 33.5</td>
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<tr>
<td></td>
<td>Night</td>
<td>23.6 ± 42.7</td>
</tr>
<tr>
<td>Red-Red</td>
<td>Daytime</td>
<td>19.1 ± 39.5</td>
</tr>
<tr>
<td></td>
<td>Night</td>
<td>29.1 ± 45.6</td>
</tr>
</tbody>
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Supplementary material

Figure S1 Arrangement of the artificial caterpillar patch during the predator exposure experiments during the summer 2015 in West Zealand, Denmark.
Article VII

Ferrante M, Barone G, Lövei GL

The carabid *Pterostichus melanarius* uses chemical cues for opportunistic predation and saprophagy but not for finding healthy prey.

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The carabid *Pterostichus melanarius* uses chemical cues for opportunistic predation and saprophagy but not for finding healthy prey

Marco Ferrante¹, Giulio Barone¹,², Gábor L. Lövei¹

¹Department of Agroecology, Aarhus University, Flakkebjerg Research Centre, Forgøsvej 1, DK-4200 Slagelse, Denmark

²Current address: Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Viale delle Scienze, Ed. 16, I-90128, Palermo, Italy

Running title: *P. melanarius* prey choice

Corresponding author: Marco Ferrante, Department of Agroecology, Aarhus University, Flakkebjerg Research Centre, Forgøsvej 1, DK4200 Slagelse, Denmark. E-mail: marco.ferrante@agro.au.dk
The carabid *Pterostichus melanarius* use chemical cues for opportunistic predation and saprophagy but not for finding healthy prey

**Abstract**

The sentinel prey method can quantify predation pressure in various habitats. Real prey is assumed to more realistically mimic the predator experience but the predator can rarely be identified. Artificial prey made of plasticine may lack real chemical cues, but provides information about predator identity. However, the relationship between predation pressure registered by artificial vs. real prey is not clear. We tested the relative attractiveness of artificial caterpillars, and intact, wounded, or dead larvae of the cabbage moth (*Mamestra brassicae*) for the carabid predator *Pterostichus melanarius* Illiger (Coleoptera: Carabidae). *P. melanarius* adults were attracted to dead caterpillars more than to live or wounded ones. Coating artificial caterpillars with caterpillar haemolymph increased their attractiveness. However, predators were not attracted more to healthy, real caterpillars than to "untreated" artificial ones. We conclude that using artificial caterpillars does not underestimate predation pressure by this carabid on healthy caterpillars.

**Keywords:** ground beetle, artificial caterpillar, choice test, insect behaviour, scavenging, sentinel prey,
Introduction

Predation is one of the main causes of mortality with a potential to limit and regulate prey populations (Hairston et al. 1960). Global estimates of species diversity and abundance suggest that most predators are invertebrates, mostly insects (Gullan and Cranston 2009). In cultivated habitats, invertebrate predators are considered useful as biocontrol agents (Naylor and Ehrlich 1997), limiting the number of herbivorous arthropods, and reducing the economic loss caused by them.

Monitoring predatory activity is important, especially for biological control, but we also need to know the predators involved, and their contribution to the overall predation pressure. Invertebrate predation can be investigated by field observations, prey labelling, and gut content identification (Greenstone 1996; Macfadyen et al. 2015; Sunderland 1987), which allow determining predator identity. However, these methods rarely provide information about the magnitude of predator activity. Exclusion methods can separate, to a limited degree, predation pressure exerted by larger groups, e.g. birds vs. small invertebrates (Mansion-Vaquié et al. 2017).

In their review on methods to quantify ecosystem functions, Meyer et al. (2015) recommend the use of sentinel prey to characterise predation intensity as an ecosystem service of natural pest control. The sentinel prey method consists of exposing a known number of prey items in the field for a given period, and estimating predation pressure from the observed rate of disappearance (Kidd and Jervis 2005). Sentinel prey are often real, but they can also be made from an artificial material, only imitating real prey. Contrarily to real prey which are often entirely consumed by
predators, artificial ones are usually not consumed, and display signs of predator attack, thus providing clues about predator identity (albeit with varying precision: Low et al. 2014).

Exposing sentinel prey to potential predators was used to study predation by vertebrates (mostly bird eggs or egg mimics (Møller 1987) earlier than on invertebrates (Lövei and Ferrante, 2017; but see also Brodie III (1993), Daly et al. (2008)). A pertinent question when using artificial sentinel prey is how closely the reaction of predators mimics their behaviour in "real" situations (Zou et al. 2017). The discussion around the use of artificial nests or eggs has thrown up serious reservations (Major and Kendal 1996; Wilson et al. 1998), so the question of realism is also relevant for the invertebrate sentinel prey method (Remmel et al. 2011; Zanette 2002).

Different versions of the sentinel prey, as well as live, wounded, or dead real prey provide different information (Furlong and Zalucki 2010; Zou et al. 2017), yet current practice often considers them equivalent as pointed out by Lövei and Ferrante (2017). A recent assessment of the sentinel method (Lövei and Ferrante 2017) concluded that used with appropriate standardisation, the artificial sentinel prey is useful for comparative studies, even at large scales. To possibly calibrate how predation pressure measured using artificial sentinels relates to predation pressure on natural prey, behavioural experiments under laboratory conditions would be useful.

We tested the reaction of the generalist carabid, *Pterostichus melanarius* Illiger (Coleoptera: Carabidae) to various kinds of sentinel prey in such experiments. We chose a ground beetle, because carabids are an important taxonomic group including many predatory species (Lövei and Sunderland 1996), which frequently attack artificial caterpillars (Ferrante et al. 2014; Mansion-Vaquié et al. 2017). We hypothesised that live prey, whether both healthy and wounded (H1), as
well as an artificial prey coated with haemolymph obtained from a real prey (H2) would be more attractive than an intact artificial caterpillar, as the last one has only the general appearance of a prey, but without natural chemical cues. Predators are often opportunistic (Wilson and Wolkovich 2011), thus we hypothesised that P. melanarius will also be more responsive to dead than live prey (H3).

In choice tests, hungry P. melanarius adults responded to haemolymph-coated artificial prey, as well as wounded or dead prey indicating an ability to perceive prey-originated chemical cues. However, they did not prefer healthy caterpillar prey to untreated artificial ones.

**Material and methods**

**Insect collection and rearing**

**Real prey**

Larvae of a polyphagous herbivore, the cabbage moth, *Mamestra brassicae* L., (Lepidoptera: Noctuidae) were used as real prey. *M. brassicae* eggs were obtained from a laboratory culture kept at the Plant Protection Institute, Budapest, Hungary.

After hatching, larvae were kept in plastic containers at 12°C, under natural photoperiod, and were fed with freshly washed pak choi (*Brassica rapa* L. subsp. *chinensis*) leaves every 2-3 days. This provided enough moisture for the larvae, so water was not separately provided. We only used the green larval morph (to match the artificial caterpillar colour, see later) in their 3rd larval stage, when they were about 15 mm long.

**Artificial prey**
The artificial prey was a 15 mm long and 3 mm thick “caterpillar”, made of light green plasticine (Smeedi plus, V. nr. 776609, Denmark), using a modified garlic press as described by Howe et al. (2009).

**Predator**

Our model predator, *P. melanarius* is a widespread Palearctic species, present also in North America (Niemelä and Spence 1999). Its prey range is extensive, including various invertebrates, alive as well as dead (Larochelle 1990). *P. melanarius* is also one of the common autumn-breeding species in Denmark (Lövei et al. 2002; Lövei et al. 2005). The time of the experiment coincided with the late activity period (Elek et al. 2017), when beetles usually feed voraciously before hibernation.

Adult *P. melanarius* were collected by live pitfall traps on the Aarhus University research farm at Flakkebjerg Research Centre near Slagelse, Denmark, during July 2016. Captured beetles were put into transparent plastic containers, filled with an approximately 1cm thick layer of sterilised soil, and kept in a climatic chamber at 25°C, 85% relative humidity, and a 16h:8h L:D photoperiod. *P. melanarius* is a night-active species, and in order to facilitate the experiments, we shifted the photoperiod of the captive beetles so that the scotophase started at 09:00 am CET (celestial time). Carabids were fed every second day with live house fly (*Musca domestica* L., Diptera: Muscidae) pupae, and had free access to water provided in a test tube closed with a piece of cotton. Up to eight beetles were placed in the same container; cannibalism was never observed. Before subjected to the choice test, individual beetles were separated, and starved for 5 days, during which time they only had access to water.
Experimental arena design

The testing arena was designed as follows: a plastic box (15cm x 15cm, 18cm height), containing a thin layer of sterilised soil, was connected with a flexible plastic tube (3.5-5.5cm diam.) to the Y-shaped glass olfactometer (4cm diameter, 20cm long main arm, two 18cm long “forks” at a 45° angle). Two horizontally positioned Falcon tubes (10 cm long, 3.5cm diam., Almeco, Esbjerg, Denmark), containing the prey, were attached to the ends of the Y-tube with parafilm. To allow undisturbed air flow through the apparatus, the bottom of each Falcon tube was cut off, and three holes (0.5 mm diam.) were cut into its lid. Air flow was provided by a suction pump (KNF Neuberger, Trenton, NJ, USA, model NMP 830 KNDC), operated at 1000 cm³ min⁻¹, connected to the plastic box, at the side opposite to the olfactometer connection. Before starting the experiment, the glass tube was immersed in 5% Deconex detergent solution for 6h, rinsed with 96% ethyl alcohol, followed by two rinsing with sterile water, and was air-dried.

Behavioural test procedure

Prey selection experiments were done under laboratory conditions, in semi-darkness. The beetle to be tested was first kept at 12°C for 5-10 min (to prevent frantic activity immediately after release in reaction to handling), then released into the arena, and the box was closed. The trial started when the beetle made its first move. A choice was recorded when the beetle reached the end of an arm (where the arm joined the tube with a prey offered). If no decision was made within 15 min, the trial was stopped. There were only 10 such cases out of 356 trial runs, and these were excluded from the analysis. We also recorded the time taken to reach a decision. At the end of a trial, a new trial run with a new, naive beetle was started.
After ten tests, the olfactometer tube was turned by 180°, which switched prey positions, and exposed an untouched side within the olfactometer tube. After 20 trial runs, the glass tube and the Falcon containers were washed first with acetone, followed by 96% ethyl alcohol, rinsed with distilled water, and dried before continuing the experiment with a new choice combination, and naïve beetles. All trials were carried out between 22 August and 16 September 2016, in the laboratories at the Flakkebjerg Research Centre of Aarhus University, Denmark. After completing the tests, the beetles were fed, and replaced in their container.

We used the following treatments, with identical-sized (15 mm) prey:

- live, unhurt caterpillar. Upon being touched, all caterpillars regurgitated some gut content. This was removed, but chemical cues may have been produced.

- freshly wounded caterpillar. A caterpillar was pierced twice on its last 2-3 segments with an entomological pin (size 00). Haemolymph spilled out of the wounds, which was not removed.

- dead, decomposing caterpillar. A caterpillar was killed by freezing at -79°C, and left for 24 h at room temperature before the test.

- intact artificial caterpillar, made of odourless green plasticine (Howe et al. 2009). Preliminary experiments indicated that beetles did not distinguish between caterpillars produced by gloved or bare hands, so no gloves were used when preparing the artificial prey.

- artificial caterpillar, coated with haemolymph of a freshly killed *M. brassicae* larva.

Immediately before the test started, a *M. brassicae* caterpillar was cut with a scalpel, and the artificial caterpillar was immersed in the caterpillar body fluid.

Due to logistical constraints, only the following choice combinations were tested:
1) live, unhurt vs. intact artificial caterpillar,
2) live, unhurt vs. dead caterpillar,
3) live, unhurt vs. wounded caterpillar,
4) live, unhurt vs. artificial caterpillar coated with haemolymph,
5) intact artificial caterpillar vs. artificial caterpillar coated with haemolymph,
6) dead caterpillar vs. artificial caterpillar coated with haemolymph, and
7) wounded caterpillar vs. artificial caterpillar coated with haemolymph.

A total of 157 adult beetles were used in the behavioural experiments. An individual beetle could
be used for a different but not the same choice test on the same day. No beetle was tested twice
in the same prey choice combination. All of them were released near their site of capture at the
end of the experiments.

Evaluation methods

The decision times of beetles selecting a prey were compared using a Wilcoxon–Mann–Whitney
test to account for non-normality. We evaluated predator preference choice using the $\chi^2$ test
(Yates 1934). All analyses were performed using the R 3.3.2 software (R Core Team, 2016).

Results

$P. melanarius$ adults used on average 134.4 sec (SD = 153.8 sec, $N = 356$) to make a choice.

There was no significant difference (Wilcoxon–Mann–Whitney test: $W = 15176, P = 0.56$)
between decision times in tests where one of the two prey treatments was significantly preferred
(mean = 152.6 sec, SD = 163.4 sec, \(N = 164\)), and when there was no preference (mean = 152.4 sec, SD = 150.1 min, \(N = 192\)).

\textit{P. melanarius} adults did not show preference for live, unhurt over intact artificial caterpillars (\(\chi^2 = 0.32, \ N = 50, \ P = 0.572\)). However, artificial caterpillars became significantly more attractive if coated with haemolymph than untreated ones (\(\chi^2 = 8.00, \ N = 50, \ P = 0.005\)). Wounded caterpillars were also significantly more frequently chosen over live, unhurt ones (\(\chi^2 = 3.93, \ N = 43, \ P = 0.047\)). Haemolymph-coated artificial caterpillars were more frequently chosen than real ones, whether live, wounded or dead but none of these differences were significant (\(\chi^2 = 0.4-1.14, \ N=23-63, \ P=0.29-0.53\)). Beetles also significantly preferred dead caterpillars over live, unhurt ones (\(\chi^2 = 4.07, \ N = 71, \ P = 0.044\)).

**Discussion**

\textit{P. melanarius} adults seem to be able to perceive chemical stimuli but use them selectively in prey choice. Live, intact real prey was not more attractive than the artificial larva which indicates that using artificial caterpillars may not distort the registered predation pressure by ground beetles on healthy, live prey. The same species, when hunting slugs, apparently does not use chemical cues to find healthy prey (McKemey et al. 2004) but pterostichine species can use olfactory cues for locating prey (Wheater 1989). However, wounded prey attracted the predator, so our H1 was partially supported. The artificial caterpillars mimicking wounded prey also attracted the model predator (but not significantly in all choices, so H2 was only partially supported).
One possible explanation is that simply detecting the presence of another living organism may not be informative enough for the predator. The perceived prey may decide to defend itself (Benelli 2015) or escape (Losey and Denno 1998), or in the worst case it could also be a predator. Being able to react to chemical cues associated with wounding is obviously advantageous, as injured prey usually has an impaired mobility (Figiel Jr and Semlitsch 1991), and thus may indicate an easy meal.

Our results confirm the opportunistic feeding behaviour in *P. melanarius* (Foltan et al. 2005), including not only being drawn to wounded, but also to dead prey. The chemistry of putrefaction has been poorly investigated (Dekeirsschieter et al. 2009), but some of the volatiles generated during the early stages of decomposition were obviously perceived. Carrion older than three days lose their attractiveness (Foltan et al. 2005), which indicates that the changing chemical profile during decay also has a role in prey selection/acceptance. This is likely for other polyphagous carabids: *P. cupreus* displays similar behaviour, also readily consuming dead aphids and springtails (Mundy et al. 2000).

Carrion is an important source of food in terms of both nutrients and availability (Carter et al. 2007; Von Berg et al. 2012), and requires a shorter handling time compared to real prey (Lang and Gsödl 2001). Many generalist predators are also scavengers (Foltan et al. 2005; Ruxton and Houston 2004). Currently available techniques to identify prey in predators cannot differentiate scavenging from other forms of consumption (Foltan et al. 2005), which makes the importance of scavenging probably underestimated (DeVault et al. 2003; Wilson and Wolkovich 2011). For example, all of the 17 predatory species of carabids collected during a study on decomposition in Czech Republic were also “accidentally” found on carrion (Kočárek 2003).
Chemical cues are an important source of information for invertebrates (Schiestl 2010; Vet and Dicke 1992). However, biology and ecology of invertebrates are extremely varied, which is reflected also in their use of the olfactory information. For example, specialised parasitoids use chemical information for finding hosts (Wickremasinghe and Emden 1992), but relevant chemical information is often specific and requires accuracy (Vet and Dicke 1992). There is no proof that chemical cues have the same importance for generalist predators (Vet and Dicke 1992), which during the search of the prey likely use also other cues (e.g. visual stimuli, vibrations, Harmon et al. 1998; Virant-Doberlet and Cokl 2004).

Nonetheless, the ability in generalist predators to perceive and process chemical information brings fitness benefits. Finding food is only one of their concerns, together with finding suitable habitats for reproduction, or avoiding being preyed (Lövei and Sunderland 1996). They certainly have the ability to react to chemical cues, but the role of chemical information in hunting may have been overestimated. Attacking anything that seems edible, or at least not threatening, may be the best low risk strategy, and the most profitable one.

This study is the first comparison of predator reaction to real vs. artificial caterpillar prey under laboratory conditions. Even though artificial caterpillars are easy to produce and manipulate (Howe et al. 2009), real sentinel prey have been traditionally preferred because they are believed to more closely resemble the real situation (Lövei and Ferrante, 2017). Our results suggest that by the use of artificial caterpillars, researchers do not under- or overestimate predation rates due to differences in chemical qualities, and that choosing real sentinel prey merely on the basis of the assumption of “more realistic features” is not necessary.
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Discussion

Article I

Artificial caterpillars can be used in various habitats and arrangements to measure predation intensity both by invertebrate and vertebrate predators. Nevertheless, the method lacks standardisation, and comparisons among studies is challenging. The most problematic aspects is the exposure period which is often not 24h (or its multiples), and when only predated caterpillars are replaced, which causes lack of independence and loss of statistical power. While the method was first used in 1994, it became popular only in the last decade, and mostly in forest habitats; data from cultivated areas are still scarce (Fig.3). Research connected to this Thesis increased the available data from agricultural habitats by adding five locations to the existing eight (red crosses on Fig.3).

Fig.3. Worldwide use of the artificial caterpillar method until April 2017. Circles: study sites in forest habitats, crosses: in cultivated habitats. Black symbols indicate published studies; data generated during this PhD research are marked with red ones.
**Article II**

Urbanisation causes habitat loss, pollution, and invasion of non-native species, with negative effects on biodiversity (McKinney 2002) and ecological processes (Faeth et al. 2005). Sampling ground beetles from the same study area, Elek & Lövei (2007) showed that urbanisation modifies carabid assemblages, and suburban sites become poorer in terms of species richness and activity density. Ferrante et al. (2014) showed that predation rates by chewing insect (26.8% of the bites), ant (10.5%), and mammal (12.1%) are also affected by urbanisation. We expected the highest chewing insect predation pressure in the least urbanised area, and lowest in the suburban one; this prediction was supported. The higher ant and small mammal predation rates in more urbanised areas indicated that certain species may be favoured by the altered conditions; others may benefit from lack of predators present in the original forest habitat (Eötvös et al., 2017).

**Article III**

Flowery margins around fields are often cited as an effective way to increase predator activity (Gurr et al. 2004) but in our experiments, planting a specially designed flower mixture around wheat fields did not increase the abundance of generalist arthropods, nor predation intensity by ground active predators. For this natural enemy group, habitat structure is probably more important than additional resources thus provided, or the extra potential prey attracted by the flowers. Most of the sentinel prey were attacked by chewing insects (88% of the bites), and likely by large (≥15mm) predatory carabids with whose abundance we found a positive correlation. Quantitative estimates of predation remain essential in order to understand its relationship with biodiversity. Attempts to correlate predator abundance or diversity with predation rate showed positive results (Speight and Lawton 1976), but others did not (Pearce and Zalucki 2006). Correlations between rates of disappearance and densities of relatively loosely defined groups such as “predatory insects” (Chang and Snyder 2004), or “ground-active predators” (O’Neal et al. 2005) do little to clarify uncertainty about the identity of the potential predators responsible for the attacks. Our results supported the idea that structure and function are positively related, but also that this relationship is complex, and neither structural nor functional data should be used as a simple proxy for the other. These results reinforced the need for directly monitoring ESs.
**Article IV**

Invertebrate and vertebrate predation rates were both affected by habitat type in the studied Argentinian landscape, and we registered more attack marks in forest fragments (41.6% d⁻¹) and at edges (44.5% d⁻¹) than in the crop (21.5% d⁻¹). The significantly higher bird predation rates in the crop occurred possibly because the less dense vegetation made prey more accessible. Fragment size was positively related to small mammal predation rates, but negatively to chewing insect predator activity, consistent with previous findings (Elliott et al. 2002; Moreno et al. 2013). Distance from the continuous forest showed another contrasting pattern: invertebrate predation increased with increasing distance from the supposed source, while vertebrate predation decreased. Differences in predator mobility and matrix permeability can both explain these results. Proximity to a fragment edge did not influence invertebrate predation, suggesting absence of spill over at small spatial scale. Invertebrate predation was significantly related to live plant ground cover, indicating the importance of factors at local scale. Crop phenology significantly influenced total predation rate, which peaked during the early milky ripening stage. Our results showed that being able to partition predation rate by predator group can be a great advantage, unmasking the contribution of different predators, which would remain undetected by registering only total predation.

**Article V**

The use of artificial caterpillars is rare in cultivated areas, and work connected to this dissertation produced data from numerous other locations (Fig.3). The average predation rate in maize was 11.7% d⁻¹, with the lowest value in Argentina (7.2% d⁻¹), and the highest in Romania (29.0% d⁻¹). Predation rate on ground caterpillars (15.7% d⁻¹) was significantly higher than on maize leaves (6.0% d⁻¹). The predation activity profiles varied, due to differences in biogeography, species pool, and agricultural practices (Kleijn et al. 2009). We found no significant differences between predation rates in Bt (12.7% d⁻¹) vs. non-Bt (11.1% d⁻¹) maize, although the small size of the plots does not allow to generalise this conclusion to landscape level. The possibility of “landscape-level surprises” is well illustrated by the findings in China for Bt cotton, where the cotton plots proved to be metapopulation sinks for the major pest, *Helicoverpa armigera*, while sources for mirid bugs (Lu et al. 2010; Wu et al. 2008). Nonetheless, results show that artificial caterpillars can be used to obtain baseline data, and to generate comparable quantitative data on predation pressure.
Both invertebrate and vertebrate predation rates on caterpillars on the ground were significantly higher during the night than daytime, which can be explained by the activity peaks of chewing insects and small mammals, the two main predator groups in this study. These results are different from those by Seifert et al. (2016) who, using sentinel prey on foliage in the Amazon rainforest, found ants to be the main predators, and predation rates higher during day- than night-time. However, caterpillars regularly occur and have to move on the ground: when changing host plants, after escape from a predator attack by dropping, after being dislodged by wind or rain, or when seeking overwintering or pupation sites. Our data indicate that the enemy-free time, claimed by Seifert et al. (2016) may not exist for caterpillars. Additionally, prey colouration did not interact with exposure time (day vs. night), indicating that the two factors are independent of each other. Invertebrate predation was significantly higher on pairs of red caterpillars than other colour combinations, while vertebrate predation was not significantly affected by prey colour. It is likely that prey colour per se has little relevance, as several vertebrates (Yokoyama and Radlwimmer 2001) and invertebrates (Salcedo et al. 2003) cannot see red colour but perceive the contrast with the background (Land 1997).

Pterostichus melanarius showed a clear ability to perceive chemical cues, and preferred wounded and dead prey to other ones offered. Wounded prey require less effort to be subdued, and this reduces the risk of being injured, and unsuccessful predation in general (Vermeij 1982). Consequently, this preference is not unexpected. Similarly, dead prey may be an easy, free meal. The chemical changes generated during putrefaction may determine the attractiveness of such prey (Dekeirsschieter et al. 2009). In the case of P. melanarius, this period seems to be three days (Foltan et al. 2005). Although these results support the idea that opportunistic feeding is common, current methods to detect arthropod predation cannot distinguish between true predation, secondary predation, or scavenging (Furlong 2015), therefore the magnitude of “real” predation remains unquantified. Lövei and Ferrante (2017) found that artificial caterpillars may underestimate predation in comparison to real prey, but the choice test results suggest that this difference is not only due to chemical attributes. Behavioural, physiological, and morphological characteristics of the prey are also important (Greeney et al. 2012), as well as the interaction between some of these factors (Hossie et al. 2015). For the general applicability of the method, it is encouraging that live, unhurt prey was not preferred over the intact artificial one.
Future perspectives and conclusions

The potential of the artificial caterpillar as a monitoring method has gained additional support by its use in various settings presented in this Thesis. However, there are additional aspects that would further enhance its usefulness.

The arrangement of sentinel prey is rarely supported by ecological arguments; it currently seems to follow logistical convenience (Lövei and Ferrante 2017). Artificial caterpillars in close proximity have greater chances to be attacked by the same predator, which could lead to an overestimation of predation rate, as well as violating the assumption of statistical independence. Autocorrelation tests may determine if, and within what distance, statistical dependence should be taken into account during analysis (Zuur et al. 2010).

Another important aspect concerns the reliability of the identification of attack marks, discussed by Low et al. (2014), whose article is currently the only available guideline to identify predatory marks on artificial caterpillars (but see also the web catalogue from Papua New Guinea by K. Sam, http://tvardikova.weebly.com/uploads/3/8/5/6/3856833/bite_guide.pdf). Controlled laboratory experiments where a known predator is exposed to the artificial caterpillars and the clear documentation of the resulting attack marks would be extremely useful. The creation of a public database with photos and descriptions of these marks would allow to increase our ability to identify predators (and non-predators), and it would likely help to spread the use of the method.

At a more basic level, the reasons why both invertebrate and vertebrate predators attack artificial caterpillars are still unclear. Plasticine is not poisonous but does not provide nutritional benefits, so it is unlikely that predators are attracted to it for its nutritional value. However, bites of insects and non-predatory small rodents found on plasticine snakes (Buasso et al. 2006; Pfennig et al. 2007) suggest that plasticine may be attractive for other reasons. Artificial caterpillars are rarely entirely consumed, and multiple bites by what is presumably a single predator are not uncommon (M. Ferrante pers. obs.). This raises further questions about predator behaviour about which, at the moment, we can only speculate. Multiple attacks may be related to the hunger level of the predator, or they may occur to reduce unsuccessful predation, which is frequent in nature (Vermeij 1982). All in all, a predator that fails to recognise a prey risks more than a predator that misidentifies a non-threatening but not nutritious “prey”: evolution may have favoured a bias towards detecting false-positives. Even under this “give it a try” strategy, artificial caterpillars would remain a useful comparative method.

Another aspect concerns the relationship between predation rate on artificial caterpillars, and
the real predation risk experienced by caterpillars in nature. It has been recognised that predation rate on sentinel prey cannot be interpreted as the real predation rate, and recreating the real situation is a near-impossibility using the sentinel prey method (Zou et al. 2017). However, faithfully recreating the predator-prey setting is not necessarily the most important aspect of the sentinel prey method, which is more suitable for comparing relative predation rates rather than for measuring absolute ones (Howe et al. 2009). Moreover, the use of real prey does not automatically reproduce the real situation, and other factors, such as the densities of both the naturally occurring vs. the sentinel prey also play an important role (Lövei and Ferrante 2017). It is unlikely that predation on sentinel prey may be converted to the real predation risk using a single approach. Every existing method to obtain qualitative or quantitative data about predation comes with its own advantages and disadvantages, which have to be considered based on the aim of the study. These methods are not mutually exclusive, and the most likely solution could be the simultaneous use of two or several methods (Birkhofer et al. 2017), as well as investigating the species which provide this function.

The artificial caterpillar method, together with the alternative methods to directly quantify predation rate, shift the attention from structural changes in the predator community to a functional approach. Both approaches are important, and provide complementary information. However, quantifying ESs by following structural changes is a well-established tradition, while the parallel use of tools for directly monitor ecological functions is not yet common. Similar attempts to focus the attention on the function rather than the structure are ongoing for another ecological service, pollination (Kremen et al. 2002; 2004), and they would be needed for other ESs (e.g. herbivory, seed dispersal) as well.

Conclusions

This thesis highlights the usefulness of the sentinel prey method to generate directly comparable, quantitative data on predation in many habitats, various countries, and settings. The use of artificial caterpillars is highly suitable to measure predation pressure, and it can also contribute to better understand predator behaviour. Correctly identifying the predator responsible for the predation activity that the sentinel prey aims to monitor, and the reasons why some predators are attracted by the artificial prey while others are not, are important requirements for the further development of this method. This research showed that many invertebrate and vertebrate predators attack artificial caterpillars, and their predatory marks can be easily identified. This is a great advantage compared to real prey, especially when the goal is to manipulate predation. Moreover, real prey are not necessarily more attractive than artificial
ones, as generalist predators likely rely on a wide range of stimuli. The high predation rates found during these field trials (up to 59.5%d⁻¹) support the theory that top-down forces are extremely important in ecosystems.

The artificial caterpillar method is a useful tool that can be a great addition to monitoring ESs. However, concentrating all our efforts on the utilitarian approach is risky (Silvertown 2015) and may even lead to accept species loss. It is worth to keep in mind the original goals of the Millennium Ecosystem Assessment: (1) synthesise our understanding of the significance and status of ESs, (2) make the world aware of their importance, and (3) influence policy in order to benefit from and protect ESs. Evidence is now accumulating that biodiversity is essential for sustainable ESs. A better way of tracking the status of ESs must include functional measures like the one used in this Thesis – but this should not be used as an excuse to allow seemingly “useless” species to go extinct. Biodiversity is important, and we should preserve it as best as we can.
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Predation is related to the beneficial ecosystem function of natural pest control. Traditionally, predation intensity was indirectly inferred by monitoring changes in predator abundance or diversity. This PhD research used artificial caterpillars made of plasticine to assess predation pressure. The method is inexpensive and easy to use, and allows predator identification. Under field conditions, artificial caterpillars are attacked by both invertebrates and vertebrates, and their use can provide quantitative estimates of predator activity, being eminently suitable to monitor predation rates under various settings.