Universidade de Aveiro Departamento de Biologia

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, Biodiversidade e Gestão de Ecossistemas, realizada sob a orientação científica do Prof. Doutor Eduardo Mateos, Professor associado do Departamento de Biologia Animal da Universidade de Barcelona e co-orientação do Prof. Doutor Amadeu Mortágua Velho Da Maia Soares, Professor Catedrático do Departamento de Biologia.

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palavras-chave

Sistemas agrícolas, herbicidas, artrópodes epígeos, identificação a um nível taxonómico elevado, margens de campos, *Podarcis bocagei*.

resumo

As práticas agrícolas têm sido associadas a perdas em larga escala a nível da biodiversidade. No entanto, elementos como as margens dos campos, são considerados importantes e com potencial para diminuir os impactes da agricultura ao promover fontes de alimento e refúgio. No entanto, os pesticidas e em particular os herbicidas podem afectar estas áreas e provocar impactes nas comunidades que dependem destas estruturas. Devido à sua sensibilidade a perturbações, os artrópodes são um grupo ideal para avaliar os impactes de pesticidas nos sistemas agrícolas. Para além disto têm um papel fundamental nas teias tróficas, constituindo a maior fonte de alimento para muitos vertebrados que habitam nestes ambientes, como a espécie de lagartixa *Podarcis bocagei.*

Neste estudo, avaliou-se o efeito da utilização de herbicidas nas comunidades de artrópodes de margens agrícolas, com recurso ao método de captura por armadilha de queda e a um método para estimar rapidamente a biodiversidade, a identificação a um nível taxonómico elevado. O estudo focou-se nas diferenças entre margens de campos com e sem herbicidas em duas estações, primavera e outono. A abundância, riqueza de grupos e a composição de guildas tróficas foram determinadas, assim como a abundância e tamanho dos artrópodes presas de *Podarcis bocagei*, a lagartixa mais comum na área.

Relativamente às diferenças encontradas entre os campos, destaca-se a ausência de um padrão negativo provocado pelos herbicidas. Na primavera os parâmetros avaliados foram, geralmente, mais elevados nas margens agrícolas tratadas. No outono o padrão que surgiu foi distinto, com um dos campos não expostos exibindo valores mais elevados para os parâmetros avaliados, sendo as diferenças entre os campos mais ténues.

Os resultados parecem indicar que alguns dos campos são mais favoráveis às populações de artrópodes, assim como às populações de lacertídeos. No entanto, em geral o tratamento com herbicidas não foi suficiente para explicar as variações encontradas nas comunidades de artrópodes. Outros factores não avaliados, como a estrutura da paisagem e do habitat e a composição florística podem ter contribuído para as diferenças encontradas.

keywords

Agricultural systems, herbicides, epigeic arthropods, higher level identification, field margins, *Podarcis bocagei*.

abstract

Since the advent of agricultural intensification that agricultural practices such as pesticide usage have become associated with large scale biodiversity losses. However, semi-natural landscape elements associated, such as field margins, are thought to benefit biodiversity and lessen the damaging effects of agriculture by providing sources of food and refuges. Nevertheless, Pesticides, and herbicides in particular may also affect these areas and consequently impact the communities that depend on these structures. Because of high diversity and sensitivity to disturbance, arthropods are ideal animals to assess impacts of pesticides in these ecosystems. Furthermore, they play essential roles in trophic webs, constituting the major diet components for many vertebrate species that inhabit these ecosystems, such as the lizard *Podarcis bocagei*.

In this study the effects of herbicides on arthropod communities of field margins were estimated, using pitfall traps and identification to a higher taxonomic level as a rapid assessment method of biodiversity. The study focused on the differences between herbicide treated and non-treated margins in two distinct seasons, spring and autumn, being abundance, group richness, guild composition, abundance and size of prey items of *Podarcis bocagei*, the most common lizard in the area, determined for all fields.

Differences were found between fields, but no clear negative effects were evidenced as a consequence of herbicidal treatment. In spring, margins of exposed fields generally exhibited higher values for the assessed parameters, while in autumn, a distinct pattern arose, with fewer differences found between communities.

Results seem to indicate that some of the fields may be more favourable to arthropod populations, as well as lacertid populations, but overall, herbicide treatment was not sufficient to explain the variation found in arthropod communities. Other unassessed factors such as landscape and habitat structure and plant community composition could be contributing to the differences found.

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Chapter I – Introduction, objectives and thesis structure

1 Introduction

1.1 Agricultural ecosystems

Agroecosystems are to some extent artificial ecosystems in which man has exerted selectivity toward species that hold some importance to human activities, such as crops and cattle, replacing in the process the flora and fauna that once existed in the natural ecosystems (Swift et al., 1996). This modification influences the interaction between biological communities and the physical environment, altering the functioning of these ecosystems and making them dependent on external inputs and human intervention (Swift *et al.*, 1996; Altieri, 1999). Thus, many properties of agroecosystems, such as composition and structure, will differ from the natural ecosystems in the surrounding landscape (Swift et al., 1996).

The biodiversity of agroenvironments will also differ from that of natural ecosystems and will depend on a variety of factors, including the age of the system, types of planted species, diversity of the vegetation in and around fields, management intensity and practices and the degree of isolation relatively to natural vegetation (Altieri, 1999). The vegetation surrounding fields is thought to be a very important factor in determining the biodiversity present in agroecosystems. Field margins are a common example of a structural element that is found around fields, providing in many landscapes the majority of semi-natural environments (Figure 1) (Marshall, 1988; Marshall and Moonen, 2002). These areas may contain several types of plant communities, ranging from typical plants of disturbed environments, to shrub, woodland, herb and even aquatic plant communities, potentially harboring a highly diverse plant community (Marshall, 2004). Characteristics of these plant communities, such as diversity, architecture and structural heterogeneity are thought to be important in defining the structure of the communities at higher trophic levels (Root, 1973; Hunter and Price, 1992). Margins are also thought to be important for the conservation of biodiversity, because plants may act as refuges, sources of food, overwintering and reproduction sites for invertebrates, particularly for species that do not persist in arable fields (Pollard *et al.*, 1974; Sotherton, 1984, 1985; Benton *et al.*, 2002; Marshall and Moonen, 2002). Vertebrate species may also benefit from these structures as refuges, breeding and feeding sites (Marshall and Moonen, 2002). In addition, margins can also act as reservoirs for natural enemies, leading to a better control of potential pests (Swift et al., 1996).

As modified ecosystems, many agroecosystems may harbor lower diversity and species richness than natural ecosystems. Nevertheless, low intensity management systems, such as home gardens, may have a biodiversity comparable or even higher than natural ecosystems (Swift et al., 1996). On the other hand, some of the most intensive forms of agriculture have been found to produce negative effects on biodiversity, because of intensive practices such as usage of synthetic pesticides, heavy machinery or low crop diversity (Freemark and Boutin, 1995; Swift *et al.*, 1996; Matson *et al.*, 1997). In fact, declines of many bird, small mammals, arable weeds and invertebrates species have been reported all over the world, with pesticides being viewed as major contributors (Swift *et al.*, 1996; Bengtsson *et al.*, 2005; Cole *et al.*, 2005; Hole *et al.*, 2005).

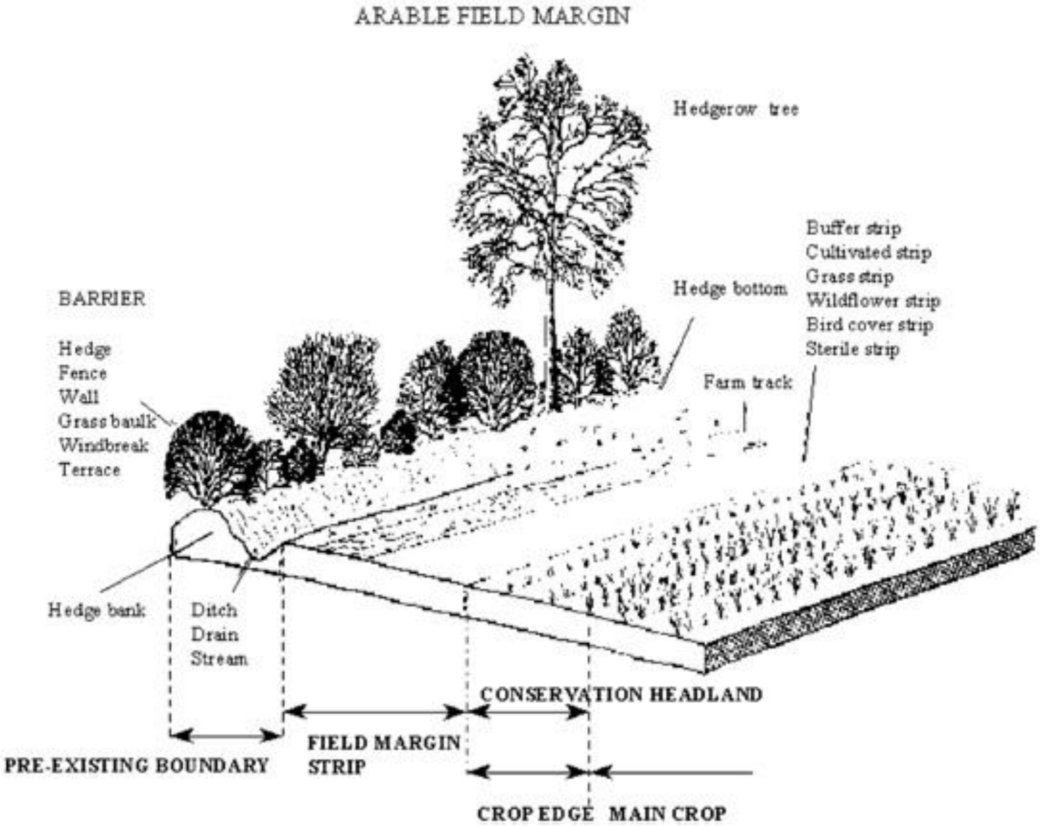

Figure 1 – The main components of an arable field margin (from Greaves and Marshall, 1987)

1.1.1 Epigeic arthropods and agroecosystems

Arthropods are the most successful extant group of animals and comprise many common organisms such as spiders, insects, isopods, millipedes and mites (Hadley, 1994). This phylum is highly diverse, containing animals exhibiting a vast array of variation on their seasonality, life cycles, feeding behavior, mobility and vegetation requirements (Southwood *et al.*, 1979; Ruppert *et al.*, 2004). In terrestrial ecosystems they comprise the most abundant animals and some, as epigeic species may be of most importance in the functioning of ecosystems (Abbott et al., 1979; Lavelle et al., 1994).

Epigeic arthropods live and feed above the ground surface and are ecologically important, generally comprising a large part of the biodiversity in agroecosystems (Abbott et al., 1979; Lavelle et al., 1994). They play several distinct roles, acting as specific or nonspecific predators, phytophagous, polyphagous, detritivorous, parasitoids and parasites (Abbott *et al.*, 1979; Marasas *et al.*, 2001; Capinera, 2010). They are important in soil and litter fragmentation as well as in decomposition and nutrient cycling processes, having influence in the soil structure, microbial processes, hydrological flows and plant productivity (Lavelle et al., 1994; Swift et al., 1996). They constitute diet components for many vertebrate groups that inhabit agroecosystems, such as reptiles, amphibians, birds and mammals, being fundamental for groups such as lizards that depend on them as major sources of food (Dominguez and Salvador, 1990; Capinera, 2010).

As a large part of the biodiversity in agroecosystems, epigeic arthropods are thought to be one of the most affected animal groups by intensive agricultural practices, in part because of the low dispersion ability of many species and their highly localized habitats (Bengtsson et al., 2005; New, 2005). These characteristics makes them very vulnerable to landscape degradation and even small changes in patches may eradicate entire populations or species (New, 2005). Impacts on these populations are thought to be caused by landscape modifications, land use intensification, as well as by the use of pesticides and other agricultural practices, such as tillage, and the reduction of semi-natural components of the landscape, such as field margins (McLaughlin and Mineau, 1995; Stoate *et al.*, 2001; Bengtsson *et al.*, 2005; New, 2005). Because of this sensitivity to environmental disturbance, as well as high diversity, small body size, high reproductive capacity and ease of sampling, these communities are considered ideal for environmental monitoring (Eyre et al., 1986; Weaver, 1995).

1.1.1.1 Pesticides effects on epigeic arthropods

Synthetic pesticides were one of the most important innovations in agricultural practices. Basically, synthetic pesticides are substances engineered to control pests, such as the ones that hinder agricultural productivity and several types exist depending on the target organism. These include herbicides, insecticides, fungicides, rodenticides, larvicides and others (McLaughlin and Mineau, 1995; van der Hoff and van Zoonen, 1999; Matthews, 2006; Hill, 2010). Impacts of these substances on organisms will depend on a variety of factors, such as application conditions, pesticide properties, the characteristics of the exposed species, application rates, physical properties of fields, climatic conditions as well as other substances applied (Holland and Luff, 2000). Since the introduction in the 1940's of more efficient and specific chemicals, pesticides have been used successfully to control undesired agricultural species (Conacher and Conacher, 1986; Hill, 2010), but have affected non-target organisms as well (e. g. Moreby et al., 1997; Wiktelius et al., 1999).

Some of the first synthetic pesticides revealed to be highly toxic for non-target organisms and highly persistent in the environment (Hill, 2010). These substances, of which DDT (dichlorodiphenyltrichloroethane) and dieldrin are examples, had the ability to accumulate in the tissues of animals which led to the poisoning of many higher level consumers and caused some populations to be severely reduced or extinct (Matthews, 2006; Hill, 2010). Following a widespread use, acknowledgement of the risks for ecosystems and biodiversity led to the banning of these pesticides from most developed countries (Hill, 2010). Nowadays, pesticides are more acutely toxic but are far less persistent in the environment, being environmental criteria an important part in the registration of the new chemicals (van der Hoff and van Zoonen, 1999; Hill, 2010). Nevertheless, effects on non-target organisms still occur, sometimes resulting in death of the organisms exposed. Sub lethal effects are also a point of concern, as they can affect aspects of the biology, such as feeding behavior, egg development, longevity or mobility, exerting some influence in the survival of the affected organisms and possibly having impacts at the population level (Desneux et al., 2007). Additionally, indirect effects such as changes in microclimate, habitat structure and food resources can also affect organisms and be important factors for biodiversity in agricultural areas (Swift *et al.*, 1996; Sánchez-Bayo, 2010).

Some groups, such as epigeic arthropods can be very susceptible to pesticides, because they live on the soil surface where contact with soil-applied pesticides is very frequent. These animals can be exposed to pesticides through inhalation, oral and dermal uptake. However, in agricultural areas one of the most important exposure mechanisms seems to be contact with pesticide coated surfaces (Van Gestel and Van Straalen, 1993).

Among the several types of pesticides, insecticides are the ones that most affect epigeic arthropods, because they are specifically designed to affect these animals. They are used in agricultural fields to control pests, but may also affect arthropods that are considered beneficial (Vickerman and Sunderland, 1977; Kevan and LaBerge, 1979; Croft and Whalon, 1982; Theiling and Croft, 1988; Wayland, 1991; McLaughlin and Mineau, 1995). Other types of pesticides such as the molluscicide methiocarb (Purvis and Bannon, 1992), the fungicide pyrazophos (Sotherton and Moreby, 1988) or even some microbial pesticides (Flexner et al., 1986) have also shown some impacts in non-target arthropods.

Herbicides, because of their scale and volume of application worldwide, are one of the major substances of concern. Although they are designed to control the growth of undesired plant species, especially weeds in cropped fields, non-target organisms such as epigeic arthropods may also be affected, either directly or indirectly (Freemark and Boutin, 1995). Some direct effects of herbicides on arthropods have been reported, even for widely used herbicides. For example, glyphosate, one of the most used herbicides worldwide, considered to be effective in the elimination of weeds, of safe usage and with few negative impacts in the environment has been found to have toxic effects on epigeic animals such as isopods and collembolans (Eijsackers, 1985; Evans *et al.*, 2010). Another widely used herbicide, atrazine, was also found to have lethal effects on collembolans, decreasing population densities on laboratory studies (Mishra, 2008). Nevertheless, direct impacts of modern herbicides on invertebrates have rarely been demonstrated in natural conditions (Sotherton et al., 1989). While some direct effects of currently used herbicides have been observed in epigeic arthropods, most of the reported effects are indirect and related to the habitat changes caused by reduction of plant species diversity and modification of physical conditions, which may have negative impacts in arthropod communities and in the communities that depend on them (e. g. Haughton *et al.*, 1999; Haughton *et al.*, 2001; Taylor *et al.*, 2006).

It is clear from the literature that there are many potential effects of pesticides in non target arthropods, but sometimes conclusions may be difficult to draw when relating laboratory with field studies, because several factors such as interaction between chemicals, physical conditions at the time of application, or even life stage of the exposed animals can influence how organisms are affected by these chemicals.

1.2 Arthropod sampling methods

The methodology used to sample invertebrates is very important when studying particular communities, as the interpretation of results depends on the information they are able to provide. Probably the most important factor to be taken in consideration is the type of organism prone to be sampled when using a certain type of trapping technique. For studying vast groups several methods should be used (New, 2005). Different techniques are available to sample terrestrial arthropods. Examples of commonly used methodologies in the study of arthropod assemblages in agroecosystems include sticky traps (Mitchell, 1963; Colunga-Garcia *et al.*, 1997; Chen and Wise, 1999; James, 2005), water pan traps (Boiteau, 1990; Duelli et al., 1999), emergence traps (Krooss and Schaefer, 1998; Mulder et al., 2000), sweep nets (Beintema et al., 1991; Meek et al., 2002), pitfall traps (Thomas and Marshall, 1999; Marasas *et al.*, 2001; Porhajašová *et al.*, 2008), suction traps (Thomas and Marshall, 1999; Brooks *et al.*, 2005) and drop cages (Pastor *et al.*, 2004; Gardiner and Hill, 2006). Although all of these methods are commonly used in agroecosystems, species assemblages captured vary greatly between them. For instance, many of the methods mentioned above have low capture efficiency for soil arthropods. Pitfalls, because of its simplicity, cheapness and ability to produce large samples are a very appealing method when compared to the others.

The following section describes the pitfall trapping technique, a common method used to sample soil arthropods.

1.2.1 Pitfall traps

Pitfall traps are among the most popular traps used to capture above ground invertebrate fauna and have been widely used since their first description by Barber (1931). This method consists essentially of a container dug in soil, semi-filled with a killing or a preservative solution, so that passing invertebrates fall in (Figure 2). Different solutions have been employed, such as water, saturated salt solutions, or ethylene glycol, and some studies have employed baits or attractant solutions to capture particular groups of interest (e.g. Greenslade and Greenslade, 1971; Waage, 1985; Sasakawa, 2007). Other studies do not use any type of solution or bait, capturing the animals alive (e.g. Mitchell, 1963). Several studies have also used hardware cloth, or other material, to avoid the falling of bigger animals and a cover to avoid over flooding of the trap by rainfall. Pitfalls have been extensively used in studies of phenology, individual abundance, diurnal activity cycles or to compare different invertebrate (Greenslade, 1964; Uetz and Unzicker, 1975; Baars, 1979; Topping and Sunderland, 1992) or vertebrate (Mengak and Guynn Jr, 1987; Friend *et al.*, 1989; Fabricius *et al.*, 2003) assemblages.

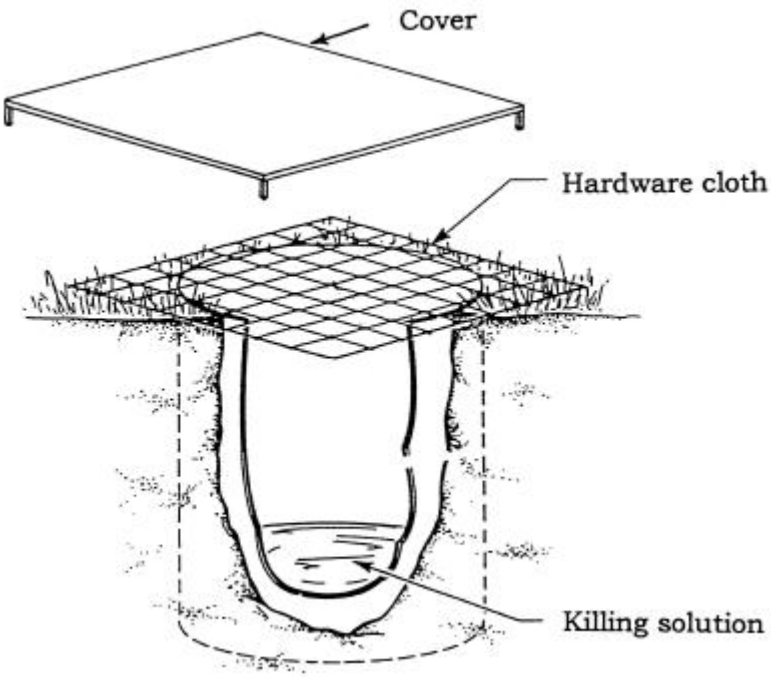

Figure 2 – Common design of a pitfall trap (from Martin, 1977)

Nevertheless, when using such method, some drawbacks must be taken in consideration by the researcher. For example, container's shape, size or constituting material can influence catches. These hinder comparability between distinct studies as distinct traps may be used (Weeks and McIntyre, 1997). In addition to the trap itself, the density of the population, the activity of the individuals, their size and behavior or seasonal activities (for example mate searching) may influence catches (Mitchell, 1963; Greenslade, 1964; Topping and Sunderland, 1992). Individual activity can also be influenced by environmental conditions, such as temperature, dryness of soil or precipitation (Mitchell, 1963; Greenslade, 1964; Joosse-van Damme, 1965).

Another important factor to be considered is habitat structure, particularly in the vicinity of the trap. Habitat structure includes the vegetation density and arrangement on the ground, as well as features of the soil structure that may affect the behavior in the proximity of the trap itself, which may affect invertebrate falling probability (Melbourne, 1999). The type of preservative fluid used in the pitfall is another source of variation, since it may have an attractive or repulsive effect towards certain species because of the color, odor, or humidity generated around the trap, thus tending to sample more of the attracted species and less of the repulsed ones (Weeks and McIntyre, 1997). Some types of fluid may even allow catches to decompose and attract saprophagous species (Porter, 2005). Finally, some experimental designs do not to use a killing agent, allowing some individuals to escape or some to prey on others that fall on the same trap (Weeks and McIntyre, 1997). The adequate type of pitfall trapping technique depends on the study objectives. If, for instance the objective is to study a group of organisms such as dung beetles, an adequate bait to attract them may be necessary (Porter, 2005), but, on the other hand, if the objective is to study a large variety of epigeic arthropod groups, the ideal is to use a technique that does not attract or repel any of them (Marasas et al., 2001).

Despite the many limitations, pitfall trapping is still a widespread methodology that yields important advantages over other methods. It is the best method to sample epigeic arthropods in studies concerning the occurrence and activity of these organisms (Greenslade, 1964). For comparisons between catches of different locations it is also a reasonable method, if factors such as the climatic conditions can be comparable and all the limitations are taken into account (Baars, 1979). Its simplicity, replicability and cheapness are also appealing characteristics of this methodology (Topping and Sunderland, 1992). They can also provide large numbers of invertebrates and sample continuously without much effort from the investigator (Waage, 1985; Topping and Sunderland, 1992). Therefore, this method is a valid and an important approach for studying soil arthropod communities.

1.3 Approaches to study arthropod communities

Monitoring of arthropod communities although invaluable is a difficult task. The high amount of arthropod species poses a serious disadvantage when trying to study such communities, given the amount of time and expertise needed to accomplish such task. This fact is partially responsible for the lesser number of studies focusing on arthropod communities as a whole compared to other groups, such as vertebrates and plants, or even a few arthropod families or orders (Shah *et al.*, 2003; Clough *et al.*, 2007; Moreno *et al.*, 2008). For that reason alternative approaches to the study of these communities have been proposed such as: (1) usage of indicator taxa, (2) restrictive instead of intensive sampling, (3) identification of morphospecies, (4) identification to a high taxonomic level, or (4) extrapolation from mathematical models, such as accumulation curves, are some of these alternative approaches (Oliver and Beattie, 1996b).

Identification to a high taxonomic level is a less demanding approach than species level identification. As it is less effort and time consuming, the identification of large amounts of samples can be accomplished much faster. Additionally it does not require highly skilled taxonomic knowledge, because identification to Order, Family or even Genus level is easier than species level identification. Thus, this approach is a less resource consuming method (Oliver and Beattie, 1996a) and has been used effectively detect differences between types of land-use at a local scale (Biaggini et al., 2007). However, some authors have argued that it is best suited for broader regional and global scales (Andersen, 1995; Hewlett, 2000; Gaston and Spicer, 2009). Even if the approach needs further validation, it is undoubtedly very useful in rapid assessment monitoring (Andersen, 1995; Báldi, 2003; Mandelik et al., 2007).

Another approach to study arthropod communities is the grouping of different taxa in trophic guilds. Trophic guilds have been defined as a set of species that exploit the same feeding resource (Simberloff and Dayan, 1991). It is a useful strategy as it divides the complex communities in more tractable units, independently of their taxonomic relation and that can be used in comparative studies between communities. While it does not regard individual species and their relations, it relies on trophic guilds with common resource use, that renders species to respond in similar ways to variations on that common resource (Adams, 1985; Simberloff and Dayan, 1991).

The following section focuses on some of the important guilds that are commonly found in agroecosystems.

1.3.1 Trophic Guilds

Predators comprise one of the important functional groups in agroecosystems, feeding on other arthropods and serving as biocontrol agents for crop pests, thus promoting the regulation of populations (Riechert and Bishop, 1990; McNabb *et al.*, 2001; Symondson *et al.*, 2002; Snyder and Ives, 2003). Some of the most commonly caught predator groups in pitfall traps are spiders, several coleopteran families, harvestman, centipedes and pseudoescorpions (Riechert and Lockley, 1984; Nentwig, 1988; DeBach and Rosen, 1991; Capinera, 2010).

Saprophagous and fungal feeder animals are also of great importance in ecosystems. Their activities are essential for the degradation of soil organic matter and the correct functioning of agroecosystems (Swift et al., 1979). These communities are usually extremely diverse, feeding on debris along with fungi, being composed by many arthropod groups such as collembolans, diplopods, isopods or termites (Giller, 1996; Beare *et al.*, 1997; Maraun *et al.*, 2003; New, 2005).

Herbivores are ecologically and taxonomically diverse and have a major role in ecosystems productivity (Huntly, 1991; Corbet, 1995). Although herbivores are generally viewed as crop enemies, because they may feed on crops, they may also act as biocontrol agents for agricultural weed species (Huntly, 1991; New, 2005). Hemipterans, orthopterans and heteropterans are some of the commonly found herbivores in agroecosystems (Gillott, 2005).

Parasitoids and parasites form a large proportion of animal diversity (Price, 1980), forming an important and decisive part in ecosystem functioning in agricultural areas by affecting other animals and plants (Marino and Landis, 1996; Thomas *et al.*, 2005). Parasitoids are defined by their larval stages which develop and feed on a single host (Godfray, 1994). These animals are economically important because they act as biocontrol agents and suppress enemy pests, being the order Hymenoptera the most prolific in number of parasitoid species among the arthropods (DeBach and Rosen, 1991; Godfray, 1994). Regarding parasites, they may affect their hosts not only through direct impacts such as mortality or reduction of fecundity, but sometimes also in behavior, affecting the probability of the host being eaten and the parasite's chance of finding another host, with the order Siphonaptera comprising some of the parasites commonly found in agroecosystems (Thomas *et al.*, 2005; Capinera, 2010).

Ants, represented by the family Formicidae (Capinera, 2010), are important herbivores, predators and scavengers (Kajak and Breymeyer, 1972; Risch and Carroll, 1982; Hölldobler and Wilson, 1990). In these ecosystems they may act as seed dispersers, affect plant productivity as well as soil processes and nutrient cycling and have been used as biocontrol agents for pests and fungal pathogens.

1.4 Objectives

Field margins are considered to be refuges for biodiversity in agroecosystems, yet herbicide usage in these field structures can lead to impacts on important communities such as arthropods which can perform many important roles in ecosystem functioning and are fundamental to many vertebrates that feed on them. For example, *Podarcis bocagei*, a lacertid lizard that inhabits agroecosystems and is the most common lizard present in the study area, depends mainly on arthropods as its source of food and is frequently found in walled field boundaries. Hence the objectives of this study are: 1 – determine the differences between epigeic arthropod communities of field margins under distinct herbicide treatments (treated and non-treated) to assess if herbicide application has negative impacts on arthropod communities; 2 – determine if arthropod feeding guilds respond differently to herbicide treatment; 3 – determine if there are differences between arthropod groups that live in field margins adjacent to walled boundaries and serve as prey items for *Podarcis bocagei* in the different fields under study and if some are more favorable than others to this species.

1.5 Document organization

This document is divided in the following chapters:

- Chapter I – Introduction, thesis structure and objectives
- Chapter II – Herbicide effects on arthropod communities of field margins
- Chapter III – Availability of lizard prey items in field margins with contrasting herbicide regimes
- Chapter IV – Discussion and Conclusion

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Chapter II – Herbicide effects on arthropod communities of field margins

Herbicide effects on arthropod communities of field margins

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Abstract

Abstract

Field margins act as shelters for invertebrate species in agricultural fields and herbicide exposure may have harmful direct or indirect effects on such communities. Epigeic arthropods were sampled in herbicide treated and untreated margins during two seasons. Organisms were identified to Family or Order level and community composition was assessed. Activity-density, species richness and guild composition were affected by sampling season, with catches and richness being higher in spring. Herbicide use did not evidence clear negative effects on arthropod communities. The predator guild was the only functional group to evidence a clear difference between treatments, exhibiting higher activity-density in spring exposed margins. Results reveal that herbicide use was not enough to explain differences found between fields and other factors such as landscape structure and plant community composition should be considered.

Keywords: Agroecosystem; Field margin; Arthropod; Herbicide; Higher taxa; Trophic guild; Pitfall trap

1 Introduction

The simplification of agricultural landscapes and the use of pesticides have been considered as one of the main causes of biodiversity loss in agricultural ecosystems (Vandermeer, 1996; Stoate *et al.*, 2001). The use of pesticides and herbicides in particular, has become a common practice worldwide, with increasing volumes and areas being treated (Conacher and Conacher, 1986; Hill, 2010). Herbicides have been generally used to control weeds in agricultural fields but can also be used in semi-natural areas within or near fields to avoid spreading of pests and weeds to the crops (Freemark and Boutin, 1995). Crop margins for example, are believed to benefit biodiversity in agricultural ecosystems since they may harbor a plant community that can support and act as a refuge for invertebrate and vertebrate communities (Pollard, 1968; Gibson and Brown, 1992; Marshall, 2004) and serve as overwintering sites (Thomas et al., 1992). These attributes are extremely important for species that do not thrive in cropped fields (Marshall et al., 2006). Field margins may also act as ecological corridors, assuring connectivity between non crop areas and preventing isolation from other important landscape patches (Altieri, 1999; New, 2005). Hence, herbicides can affect not only the biodiversity present in fields but also in these semi-natural areas.

Some of the organisms that inhabit semi-natural areas may be affected by herbicides, including non-target organisms such as above ground animals (Eijsackers and Quispel, 1988). Direct toxic effects of these substances have been shown for some arthropod groups, such as lethal effects, delayed growth or behavioral changes (Freemark and Boutin, 1995; Desneux *et al.*, 2007). However, direct effects of modern herbicides on invertebrates have rarely been demonstrated in natural conditions (Sotherton et al., 1989). Nevertheless, several studies have demonstrated indirect effects of herbicides on epigeic arthropods in agricultural areas, namely through modifications in soil physical conditions and vegetation structure (e.g. House *et al.*, 1987; Haughton *et al.*, 1999).

Herbicides may therefore influence the functioning of ecosystems through effects on epigeic arthropods, which constitute essential elements of terrestrial ecosystems and fulfill a wide variety of ecological roles (Abbott et al., 1979; Swift et al., 1996). For example, many phytophagous species, have important economical implications in agriculture, as they may act as pests and have become the target of insecticides and other types of management regimes (New, 2005). Others, such as predator or parasitoid species, are viewed as beneficial and attempts to preserve or introduce them as biological control agents in agroecosystems have been common (Oraze and Grigarick, 1989; Asteraki, 1993; Starý and Gerding, 1993). Finally, others are considered fundamental in the decomposition process and cycling of nutrients (Paoletti and Hassall, 1999), and thus essential for correct functioning of agricultural ecosystems (Swift et al., 1979). Overall, arthropod communities are fundamental to ecosystems and considered to be very sensitive to environmental changes, which makes them an ideal group for environmental monitoring (Eyre et al., 1986).

In this paper we focused on sampling of arthropod communities of field margins with pitfall traps. To allow analysis of a wide variety of arthropod groups, identification was carried out to a high taxonomic level, because this approach is faster and less expensive when compared to species level identification. In fact, surveys including a wide taxonomic spectrum are not very common. This is mostly explained by the high species number even in temperate regions, which would involve a strenuous sampling and identification effort, leading to a high cost procedure (Oliver and Beattie, 1996). One of the advantages of this method is the nonnecessity for highly skilled taxonomists, as the separation of organisms into Families or Orders is relatively easy and fast (Basset et al., 2004; Biaggini et al., 2007). Additionally, sites can be classified by grouping taxa in guilds based on a common characteristic, such as feeding habits (e.g. Clough et al., 2007), which may reveal patterns that otherwise would not be evident. This is based in the assumption that animals which share a common resource will similarly respond to variations in that resource (Adams, 1985). Also, pitfall trapping is one of the most common methods to sample epigeic arthropods (Greenslade, 1964; Thomas and Marshall, 1999; Ward et al., 2001), a technique also employed for sampling other animal groups such as mollusks (e.g. Melbourne et al., 1997) or vertebrates (e.g. Fabricius et al., 2003). Despite its shortcomings, many field surveys still rely on this method because of its simplicity, cheapness, little effort, capacity of collecting many distinct arthropod groups and adequateness for same habitat comparisons (Topping and Sunderland, 1992; Weeks and McIntyre, 1997).

Given that epigeic arthropods may be affected by herbicides applied to field margins we addressed the following questions: 1 – Do herbicides negatively affect arthropod groups in field margins? 2 – Do feeding guilds respond differently to distinct herbicide application in field margins?

2 Materials and Methods

2.1 Study sites and experimental design

Six geographically close sites with different herbicide regimes were selected. Fields were located in Northwestern Portugal in the municipalities of Vila do Conde (41°19'N, 8°40'W – exposed fields Exp 1, Exp 2, Exp 3 and Exp 4) and Vila Nova de Famalicão (41°26'N, 8°30'W – reference fields Ref 1 and Ref 2). Exposed fields had maize crops (*Zea mays* L.) in rotation with annual ryegrass (*Lolium multiflorum* Lam.), where a mixture of pesticides has been applied routinely over 30 years. The reference fields were represented by a pasture (not heavily grazed) and a farming field (several cultures) with no history of pesticide application. Annual mean temperature averages between 12.5-15 °C, total annual precipitation is 1400-1600 mm, whereas insolation is 2400-2500 h for exposed and 2300-2400 h for reference fields (Atlas do Ambiente, 1995). Soils are humic cambisols. Fields are bordered by a small vegetated margin of spontaneous grasses of variable width (from 20 cm to 1 m) and a stone wall, covered at some points with climbing plants. For the duration of our study, only herbicides were applied to exposed fields. The herbicides Spectrum™ (active ingredient (AI) – dimethenamid), Montana® (AI - glyphosate) and Controler T (AI - alachlor and terbuthylazine) were applied prior to the emergence of the crop, while Laddok Plus® (AI - dicamba and bentazon), Roundup® (AI - glyphosate) and Callisto® (AI - mesotrione), were applied after the emergence of the crop and weeds. In 2008 the herbicides applied were Spectrum™ in fields Exp 1, Exp 2 and Exp 4, Laddok Plus® in fields Exp 1 and Exp 4, Roundup® in field Exp 1, Controler T in field Exp 3 and Callisto® in field E3. In 2009 the following substances were applied: Laddok Plus® in fields E1 and E4, Montana® in field Exp 1 and Exp 4 and Callisto® in field Exp 3. A further description of the collection sites, including soil-pesticide profiles can be checked at (Amaral *et al.*, under preparation).

2.2 Arthropod Sampling

Arthropod sampling was carried out during 10 straight days in autumn (November 2008) and spring (April 2009). Non rainy days were selected when possible, to avoid biased results. Surface active arthropods were collected using pitfall traps (8 cm diameter). Ten traps were placed in each field margin close to the stone walls with a two meter spacing between them. Traps consisted of plastic containers dug into the soil, with the lip just a little bellow the ground surface. To prevent the entrance of small vertebrates, a 30 mm mesh wire piece was used and fixed with staples. Covers were positioned 20-30 mm above the trap to prevent flooding by rainwater. Traps were partially filled (1 to 2 cm) with a saturated salt solution to trap and preserve invertebrates through the collection period. After the arthropod sampling, traps were filled with ethanol (70%) and taken to the laboratory. Each sample was sieved using a 0.20 mm pore mesh and invertebrates sorted from the debris and preserved in a 70% ethanol solution until further analysis. Some of the pitfalls were not recovered from the fields, because of their destruction or filling with debris as a result of farming work. This was the case of one trap from fields Exp 2, Ref 1 and Ref 2 in autumn and one from fields Exp 1 and Exp 2 in spring, leaving some fields with only nine samples.

2.3 Arthropod processing

Invertebrates were identified to Family or Order level and counted under a stereo microscope. Adult and immature individuals were placed in distinct groups as a result of possible differences in resource utilization. As a certain degree of uncertainty existed regarding the correct identification for some larvae, these individuals were placed in groups designated by letters: for Coleoptera A, B, C, D, E, F, G, H, I; for Lepidoptera X, Y, Z; and for Diptera M. Some individuals of the Order Siphonaptera and larva of the Sepsidae Family were excluded to avoid bias in the data caused by the extremely high abundance of these groups in the pitfalls were vertebrates had fallen. Specimens that could not be identified as a result of damage or taxonomic uncertainty were excluded from further analysis.

Throughout this paper the expression group will be used to designate the set of different Families, Orders, larva and nymphs identified. Nomenclature and taxonomy of all groups was based on Barrientos (2004).

Arthropods were classified into one of five different guilds, herbivores (Her), predators (Pre), saprophagous / fungal feeders (Sap), parasitoids / parasites (Par) and ants (Ant) based in their different feeding habits. In the case of ants a separate guild was created because of the many functions that these animals may have in ecosystems: herbivores, predators or scavengers (Hölldobler and Wilson, 1990). Because individuals were assigned to Order or Family (group) level and feeding habits may vary between species assigned to a group, guild classification was based upon the major function of the respective group and was based on literature review. Individuals that could not be assigned to any of the guilds were excluded from this analysis (1.22% of individuals).

2.4 Data analysis

A $\log_{10}(x+1)$ transformation was applied to the data whenever necessary. Resemblance matrices were calculated using Bray-Curtis similarity measure and non-metric multidimensional scaling (nMDS) graphs were plotted. Similarity percentages (SIMPER) were calculated to determine which groups most contributed for the differences between seasons. Diversity indices (Shannon, Simpson and Pielou's evenness) were also computed for each of the fields. These analyses were performed using PRIMER 6 (Clarke, 2003). Individual based rarefaction curves were computed for each field using EcoSim software to allow number of groups comparison independent from the number of individuals captured (Gotelli and Entsminger, 2009). Analysis of variance (ANOVA) was performed to determine differences between fields and to compare between treatments and seasons. For statistically significant differences, Tukey's *post-hoc* tests ($P < 0.05$) were used to separate different groups. These analyses were performed using the Statistica 7 software (StatSoft, 2004).

3 Results

3.1 Abundance and community composition

A total of 9310 individuals were identified belonging to 152 different groups (mean ± SE catch per trap = 81.0 ± 6.8 individuals, n= 115), comprising 31 distinct Arthropod Orders. The most abundant Order was Hymemoptera followed by Coleoptera, Entomobryomorpha, Araneae and Isopoda comprising 39.08%, 14.40%, 13.82%, 7.55% and 5.27% of the arthropods captured, respectively. The remaining Orders comprised only 19.87% of the catches. Regarding Families (excluding larva identified with letters), Coleoptera exhibited the largest number with a total of 35, followed by Diptera with 24, Hymenoptera with 15 and Araneae with 12.

The SIMPER analysis showed that Formicidae, Porcellionidae, Entomobryomorpha, Histeridae, Gnaphosidae; Scarabaeidae, Scelionidae, Chtoniidae and Diapriidae were, in this order, the groups that contributed most to the differences between seasons. All divergent groups were found in higher numbers in spring. A two-way ANOVA evidenced that activity-density varied with season but not with treatment, while group richness varied with both season and treatment. For activity-density and group richness a significant interaction was found between treatment and season. Both parameters were higher in spring (Table 1).

Table 1 – Two way ANOVA (Season x treatment) for activity-density (Log x+1) and group richness (seasons: spring and autumn; treatments: herbicide exposed and reference).

Source of variation	d.f.	Activity-density			Group richness		
		Mean square	F	p	Mean square	F	p
Treatment	1	0.1805	2.915	0.09	332.33	10.277	0.002
Season	1	6.4661	104.448	< 0.001	1554.53	48.071	< 0.001
Season x Treatment	1	1.0750	17.365	< 0.001	631.09	19.515	< 0.001
Error	111	0.0619			32.34		

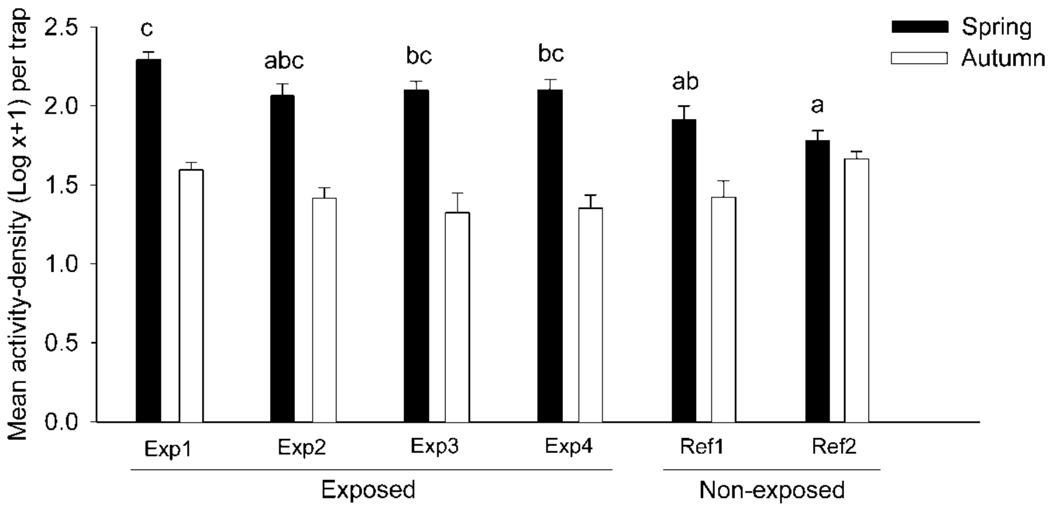

Figure 3 – Mean activity-density per trap and corresponding standard errors in autumn (2008) and spring (2009). Fields are compared within each corresponding season. Distinct letters between fields indicate statistical differences.

3.2 Spring

In spring, a total of 7904 individuals were captured with number of individuals per trap varying between a maximum of 429 and a minimum of 31 individuals. The total number of groups caught was 136 and varied between 38 and 10 groups per trap. Regarding Orders, Hymenoptera was the most captured with nearly 45 % of the catches, mainly caused by high abundances of the family Formicidae. Catches were dominated by a small number of Orders, with 95% of catches being represented by Hymenoptera, Entomobryomorpha, Coleoptera, Araneae, Isopoda, Hemiptera, Diptera, Pseudoescorpiones and Microcoryphia. The remaining 21 orders comprised approximately 5% of the catches.

Considering total catches, activity-density per trap was significantly higher in exposed fields than on field Ref 2, except field Exp 2 which did not evidence significantly higher catches (Figure 3, ANOVA Site: $F_{1,5} = 6.728$, $P < 0.001$). Mean group richness per trap also varied, with exposed fields having significantly higher values than reference fields with the exception of field Exp 3 (Figure 4, ANOVA Site: $F_{1,5} = 18.029$; $P < 0.001$).

Table 2 – Diversity indices computed for each of the fields in both sampling seasons, autumn and spring. J' - Pielou's evenness, H' – Shannon diversity index ($\log_e$), D – Simpson diversity index. Distinct letters between fields indicate statistical differences.

Field	Exp 1	Exp 2	Exp 3	Exp 4	Ref 1	Ref 2	P
Spring							
J'	0.76 ± 0.04	0.72 ± 0.04	0.67 ± 0.04	0.65 ± 0.04	0.67 ± 0.04	0.76 ± 0.04	0.2
H'	2.69 ± 0.13 b	2.38 ± 0.13 ab	2.00 ± 0.12 a	2.07 ± 0.12 a	1.91 ± 0.12 a	2.19 ± 0.12 ab	< 0.001
D	0.87 ± 0.04	0.82 ± 0.04	0.77 ± 0.04	0.73 ± 0.04	0.73 ± 0.04	0.80 ± 0.04	0.1
Autumn							
J'	0.89 ± 0.02	0.89 ± 0.02	0.89 ± 0.02	0.93 ± 0.02	0.87 ± 0.02	0.88 ± 0.02	0.42
H'	2.48 ± 0.13 ab	2.20 ± 0.14 ab	2.05 ± 0.13 ab	2.37 ± 0.13 ab	1.95 ± 0.14 a	2.60 ± 0.14 b	<0.01
D	0.91 ± 0.02 ab	0.89 ± 0.02 ab	0.90 ± 0.02 ab	0.93 ± 0.02 b	0.84 ± 0.02 a	0.92 ± 0.02 ab	0.04

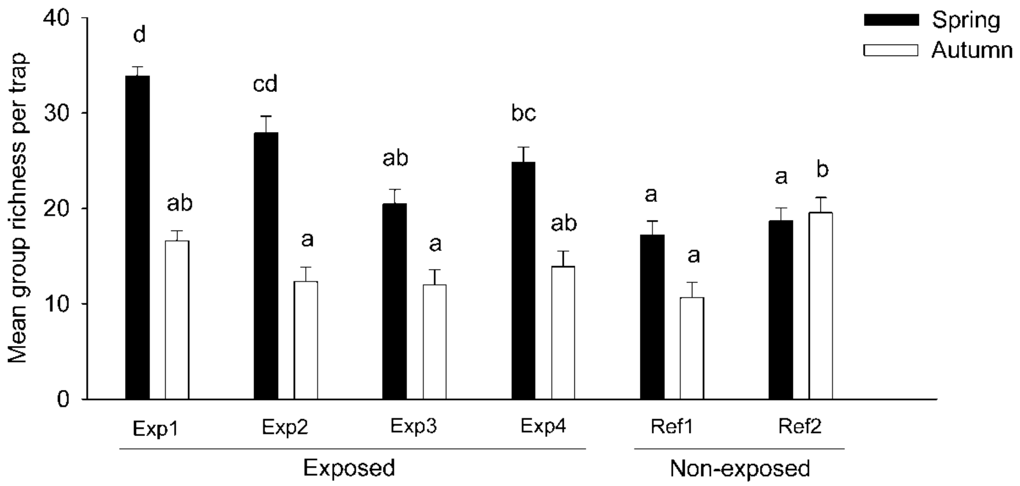

Figure 4 – Mean group richness per trap and corresponding standard errors in autumn (2008) and spring (2009). Fields are compared within each corresponding season. Distinct letters between fields indicate statistical differences.

Rarefaction curves indicate that there is higher species richness in field Ref 2 and lower species richness in field Exp 3 (Figure 5). Shannon diversity index showed significant differences between fields and evidenced that field Exp 1 was the most diverse. Simpson diversity index and Pielou's evenness did not evidence any statistically significant difference (Table 2).

Regarding arthropod communities, differences between fields were also found, namely exposed fields and field Ref 1 showed higher similarity, while field Ref 2 exhibited a distinct community (Figure 6 - a).

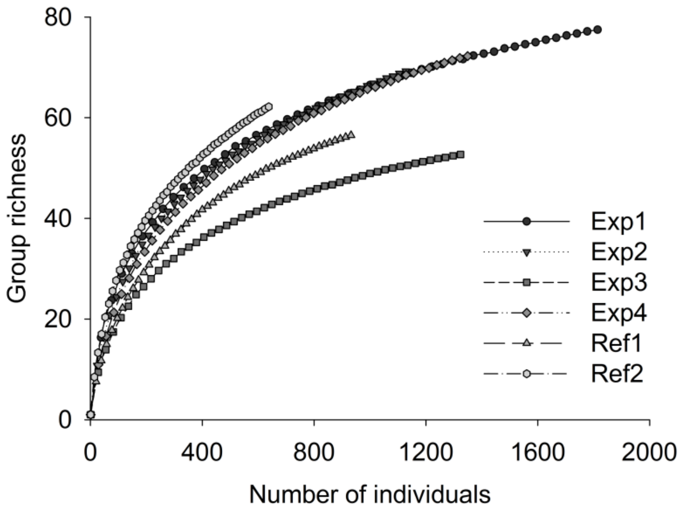

Figure 5 – Rarefaction curves of groups trapped per field in spring (2009).

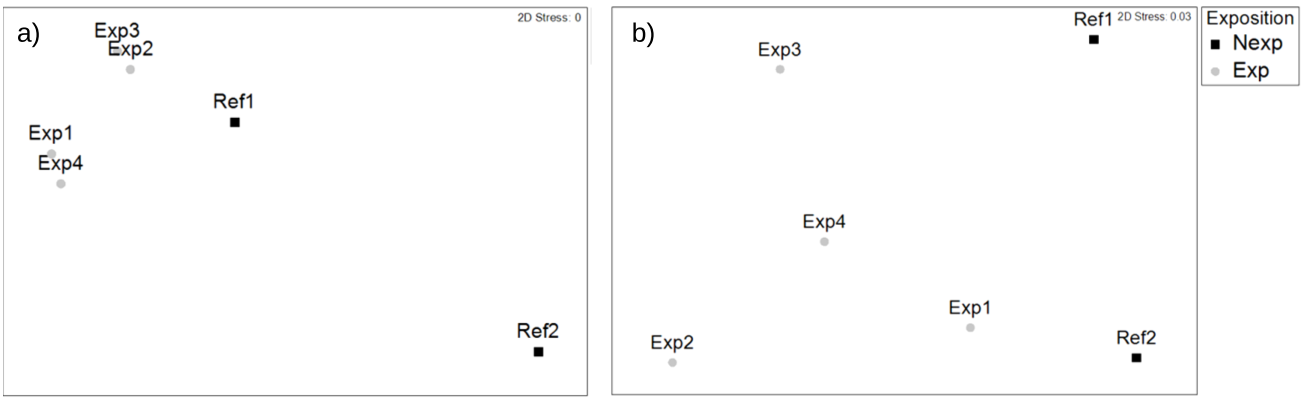

Figure 6 – Non metric multidimensional scaling (nMDS) representing arthropod assemblages of field margins: a) in spring; b) in autumn. Nexp= reference fields; E= exposed fields.

3.3 Autumn

In autumn, 1904 individuals were caught and pitfall catches varied between a maximum of 86 and a minimum of two individuals per trap. The total number of groups caught was 102 and catches varied between 28 and two groups per trap. In this season Hymenoptera was the most captured Order.

Considering groups, some differences were found between fields. Catches were higher in Ref 2, being differences statistically significant (Figure 3, ANOVA Site: $F_{1,5} = 2.601$, $P = 0.036$), but not detected with a Tukey's post hoc test. Group richness also varied significantly between fields, with site Ref 2 also having the highest group richness (Figure 4, ANOVA Site: $F_{1,5} = 4.67$; $P < 0.01$). In contrast, rarefaction curves seem to point that field Exp 4 has the highest species richness (Figure 7).

Shannon and Simpson diversity indexes pointed significant differences between fields, both evidencing Ref 1 as the least diverse field. However, Shannon index indicates that field Ref 2 is the most diverse while Simpson index indicates that Exp 4 is the most diverse field (Table 2). Arthropod communities seem to differ between fields, but in this season field Ref 2 seems to be more similar to some of the exposed fields than to field Ref 1 (Figure 6 - b).

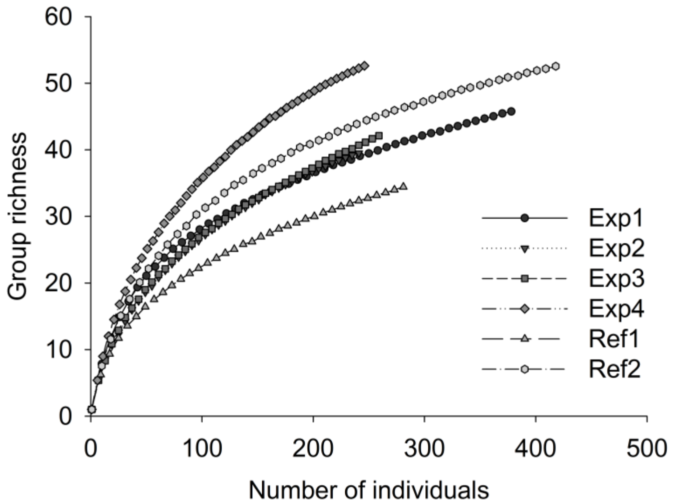

Figure 7 – Rarefaction curves of groups trapped per field in autumn (2008).

Table 3 – Number of different groups and activity-density percentages in each guild for spring and autumn. Total number of groups and activity density percentages are also represented.

Guild	Spring		Autumn		Total	
	Number of Groups	Percentage of Individuals	Number of Groups	Percentage of Individuals	Number of Groups	Percentage of Individuals
Ants	1	37.1	1	5.6	1	30.6
Herbivores	30	9.1	23	7.8	32	8.8
Parasitoids and parasites	10	7.3	11	14.4	14	8.8
Predators	39	17.7	24	34.7	43	21.2
Saprophagous and fungal feeders	46	28.8	34	37.5	50	30.6

3.4 Guild composition

In total, 2832 ants, 2815 saprophagous / fungal feeders (Sap), 1949 predators (Pre), 812 herbivores (Her) and 808 parasitoids / parasites (Par) were caught. Total number of groups caught varied between guilds, being highest in saprophagous / fungal feeders and lowest in parasitoids / parasites guild. It should be taken in consideration that for ants, the number of groups is only one (Formicidae) as a result of guild assignment methodology. Some variation also existed between seasons, but number of groups found for each guild was generally higher in spring, except for the parasitoid / parasite guild (Table 3). Guild communities varied between seasons, with a clear separation between spring and autumn fields except for spring field Ref 2 which showed a higher similarity with autumn exposed fields. Also, spring samples of field Ref 1 evidenced a high similarity with exposed fields of the same season. Autumn fields revealed a high degree of separation between exposed and reference fields (Figure 8).

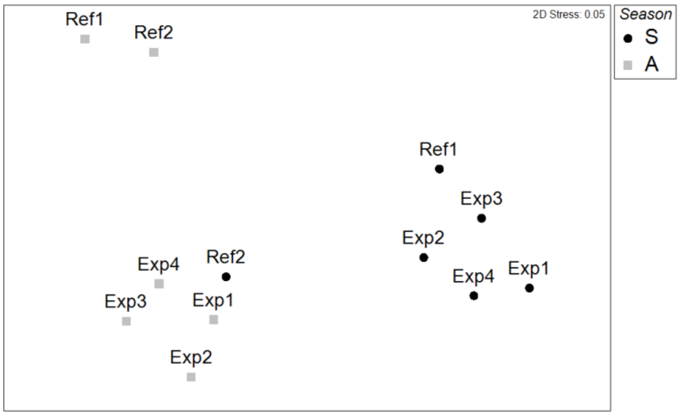

Figure 8 – Non metric multidimensional scaling (nMDS) representing guild similarities between fields (Exposed fields: Exp 1, Exp 2, Exp 3, Exp 4; Reference fields: Ref 1, Ref 2) and seasons (S = spring; A = autumn).

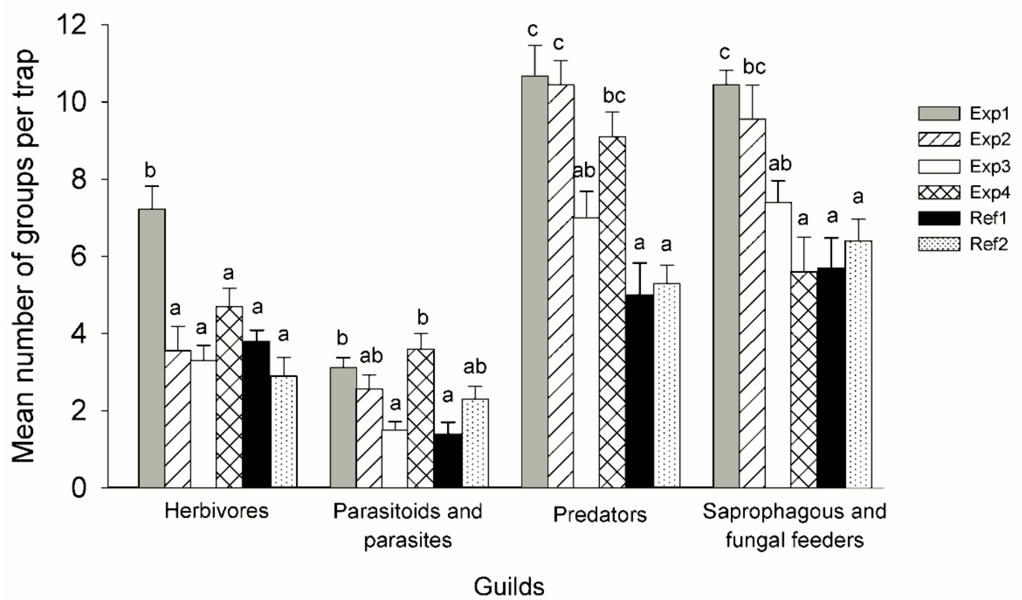

Figure 9 – Mean number of groups per trap per guild (except ants) and corresponding standard error in spring (2009). Distinct letters between fields indicate statistical differences.

In spring the fields harboring most groups was field Exp 1 for Herbivore, Predator and saprophagous / fungal feeder guilds and field Exp 4 for Parasitoid / parasite guild (Figure 9). Significant differences in activity-density and number of groups per trap between fields were found for all guilds, but differences were not consistent among and between treatments. Predator guild was the only displaying significantly higher values for activity-density in all exposed when compared to reference fields (Figure 10).

In autumn only herbivores did not evidence significant differences between fields regarding activity-density and number of groups per trap. None of the guilds evidenced significant differences between all of the exposed fields and the reference fields (Figure 11). For mean activity-density per trap also none of the treatments evidenced significantly higher values than the other (Figure 12).

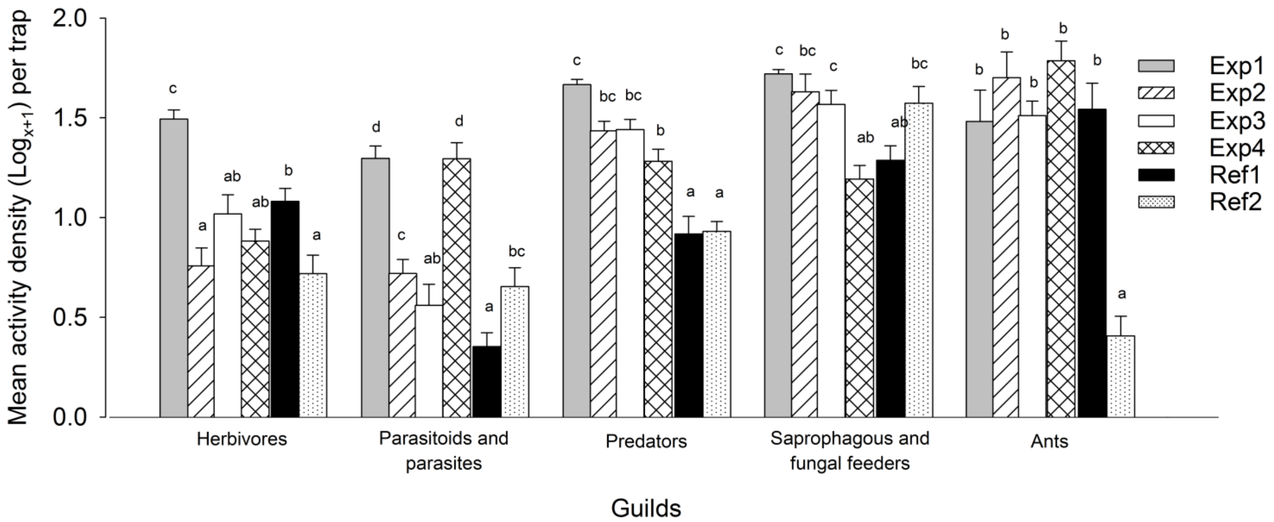

Figure 10 – Mean activity-density per trap (Log x+1) per guild and corresponding standard error in spring (2009). Distinct letters between fields indicate statistical differences.

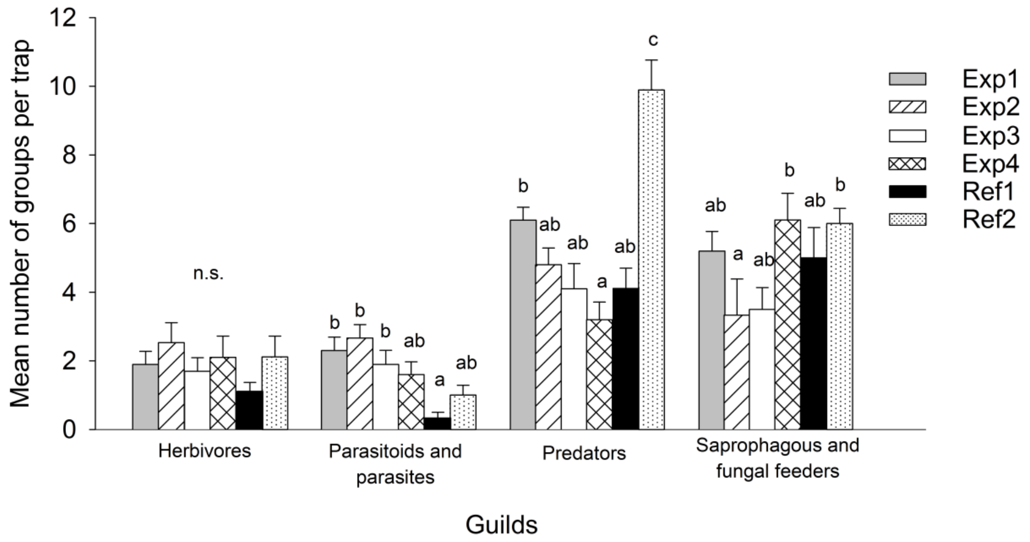

Figure 11 – Mean number of groups per trap per guilds (except ants) and corresponding standard error in autumn (2008). Distinct letters between fields indicate statistical differences. n.s. = non significant.

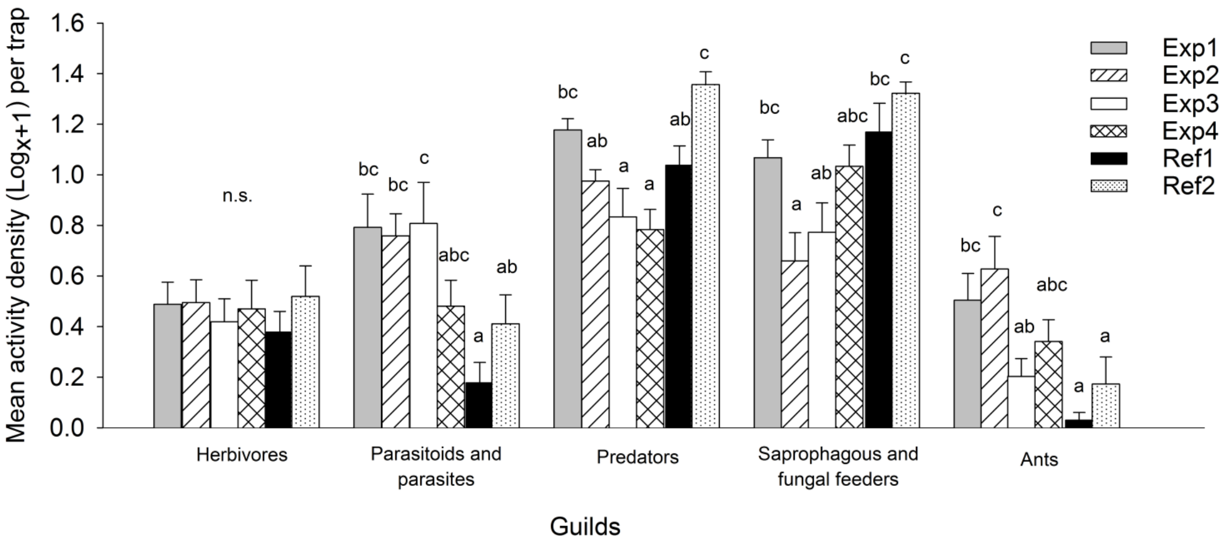

Figure 12 – Mean activity density per trap (Log x+1) per guild and corresponding standard error in autumn (2008). Distinct letters between fields indicate statistical differences. n.s. = non significant.

4 Discussion

Field margins harbor a wide variety of arthropod groups (e.g. Baines, 1990; Thomas and Marshall, 1999; Meek *et al.*, 2002). As a whole these communities can be very sensitive to changes in their habitats and modifications in community structure can be indicative of disturbance (Eyre *et al.*, 1986). Hence, monitoring of such communities can be of value in the assessment of environmental impacts on agroecosystems. In this study, arthropod guilds and communities of margins with distinct herbicide regimes were compared in two distinct seasons. Communities were expected to differ between treatments and herbicide application was expected to have detectable negative effects on arthropods. Communities were also expected to vary between seasons.

Regarding seasonal patterns, results evidenced higher richness and activity-density in spring, pointing to higher activity levels of epigeic invertebrates in this season, as was expected as a consequence of the resulting variation of climatic factors in a Mediterranean region. Regarding pesticide exposition, our results did not show a clear pattern, with exposed fields having generally higher activity-density and richness of arthropod groups in spring, but not significantly higher for all exposed fields. However, the diversity indexes and rarefaction curves do not point in the same direction, and do not evidence the reference fields as the least diverse. In autumn one of the reference fields had higher values for activity-density and richness of arthropods but not always significantly higher. Diversity indexes and rarefaction curves evidenced that the other reference field is the least diverse. In general, field communities were similar between exposed fields, but reference fields did not seem to evidence a high degree of similarity. Regarding guilds, results were not very clear between treatments, despite the significant differences between fields. Only predators were significantly more abundant in exposed rather than in reference fields in spring.

Despite both reference fields both not having herbicidal treatment, results seem to evidence that these fields have somewhat distinct communities. For both sampling seasons, communities did not seem to be very similar, with communities of field Ref 1 being more similar to those of the exposed fields in spring and communities of field Ref 2 being more similar to some of the communities of the exposed fields in autumn. Guild results also evidenced some differences with mean activity-density being distinct in spring between reference fields for herbivores, parasitoids / parasites and ants. In autumn differences in activity-density as well as for mean number of groups per guild were only evident for predators. These differences may be related to the type of land-use, because field Ref 1 is a cropped field, while field Ref 2 is a pasture. Although sampling was made in field margins with natural regeneration plant communities and not in the fields itself, in accordance with Asteraki *et al* (2004) the type of crop seems to be an influencing factor to arthropod communities of field margins. Between exposed fields, communities seem to be much more similar, with high similarities in both seasons and in guild results, despite some differences in guild richness and activity-density.

While our results do not seem to evidence negative effects of herbicides, some are known to have toxic effects on a number of invertebrate species (Jepson 1989). However, mortality under natural conditions is a rare phenomenon (Sotherton *et al.*, 1989). There are very few studies concerning the impacts of the herbicides applied in our study sites, with the exception of glyphosate. Nevertheless, some information on their effects concerning arthropods exists. For example, bentazone has been tested in laboratory, semi-field and field conditions and no harmful or little effects have been found in a number of arthropod groups including Hymenoptera, Diptera, Coleoptera, Dermaptera, Heteroptera and Araneae species (Hassan et al., 1994). Dicamba showed no effects on Carabidae (Swaminathan and Isaichev, 2000), while terbuthylazine was actually found to modify the microarthropod community by direct toxicity, as well as by indirect effects (Salminen et al., 1997). Evans et al (2010) found some evidence of behavioral changes and a reduction in long term survival in the spider *Pardosa milvina* as a result of glyphosate exposition. For alachlor, the only available study is in the aquatic environment where it was found to have only slightly toxic effects on *Chironomus riparius* (Buhl and Faerber, 1989). Added to these possible toxic impacts, indirect effects of herbicides are also expected to affect arthropod communities (e.g. Moreby and Southway, 1999; Thomas and Marshall, 1999; Denys and Tscharntke, 2002; Asteraki *et al.*, 2004). Nevertheless, no clear evidence of modification on arthropod communities was found as a result of the possible changes caused by herbicides in margin plant communities.

The abundance of ants in some of the samples might be explained by the sampling method used, because it might depend on factors such as size of the colony or its distance to the trap (Greenslade, 1973). Of all guilds considered, Predators were the only guild that seemed affected by herbicide treatment in all the studied fields during spring, as higher activity-densities were found in all exposed fields when compared with reference fields. Although surprising, higher predator activity-density is in accordance with the work of Clough et al. (2007), that found that Staphylinidae predatory species were actually more abundant in conventionally managed fields than in organic fields. Clough et al. (2007) interpret these results stating that higher predator numbers might be related to abundances of prey species that could be higher in exposed margins. In our results, such explanation is plausible because in spring the exposed margins evidenced high activity-density for all arthropod guilds. Nevertheless, when considering the results for functional groups, we should keep in mind that the sensitivity of the assignment is to be taken in consideration (Stork, 1987) and in this case it might be compromised by the taxonomic resolution.

In our study, the higher taxonomic level approach allowed us to distinguish communities and their parameters among fields, but it might not have been sufficient to discriminate finer scale differences between sites

(Cardoso et al., 2004; Mandelik et al., 2007). Vegetation structure can also influence results, because vegetation density can present movement obstacles and influence community sampling (Greenslade, 1964). However, if some differences are caused by distinct vegetation density, richness analysis can also be considered to be less prone to error when compared to activity-density, as it is based on the presence of the taxa rather than on their abundance (Noordijk et al., 2010). Nevertheless, group richness results in our study actually evidenced higher group richness of exposed fields in spring, and in autumn higher richness in only one of the reference fields. Other possible explanation to differences found between fields may be related with plant communities of margins and walls. This parameter was not quantitatively assessed, therefore actual differences are unknown. Still, margins and walls of exposed fields had an abundant flora at some points of the year, despite herbicide application and residues in fields (see Amaral et al unpublished for soil pesticide analysis).

Finally, some studies have found that landscape structure can be of greater importance than management system in determining the types and structure of communities in a given field (e.g. Weibull et al., 2000; Weibull et al., 2003). However, few studies have evaluated both factors together, being difficult to conclude which one influences diversity more (Weibull et al., 2003). Nevertheless, arthropod communities may be affected by many local and landscape factors that play roles in defining communities and their functional structure (Schweiger *et al.*, 2005).

In conclusion, despite our study not evidencing clear negative effects of herbicide application, herbicide exposition should not be disregarded as an influencing factor, although the above mentioned factors not assessed might be confounding our results. Also, significant differences were found between fields with contrasting results between seasons. Results are not consistent when considering only herbicide application, except for the activity-density of predators in spring. Apparently, other factors like landscape structure or margin plant cover and richness, which were not quantitatively analyzed in this study, might explain some of the differences found in our study.

Acknowledgments

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Chapter III – Availability of lizard prey items in field margins with contrasting herbicide regimes

Availability of lizard prey items in field margins with contrasting herbicide regimes

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Abstract

Arthropods are the main source of food in agricultural ecosystems for many vertebrates, including lacertid lizards. Herbicide usage in field margins may affect arthropod communities and the resulting changes may affect lizard populations. The present study focused on analysis of arthropod groups serving as prey items for *Podarcis bocagei*, an abundant lizard in agroenvironments from NW Portugal whose diet is well-known. Arthropods were sampled with pitfall traps in herbicide treated and untreated field margins in spring and autumn. Activity-density and size of prey were measured. Overall differences were found between fields. However, no clear differences in treatments existed for either prey groups or prey sizes. Patterns of availability varied between seasons, with some fields having higher prey availabilities on one season but not in the other. These results seem to indicate that some fields may be more favorable to lizards, although herbicide treatment was not a predictor for this pattern. Other unassessed factors such as plant structural diversity or landscape structure could be contributing to the differences found. Ultimately, higher availability of prey items may be a factor favoring lizard populations.

1 Introduction

Lacertidae is the lizard Family with the highest number of species in the Mediterranean area (Cox *et al.*, 2006), constituting a substantial part of the terrestrial fauna in Southern Europe and Northern Africa (Avery, 1978). Lacertids occupy intermediate positions in trophic webs, being preyed upon by many carnivorous vertebrates, such as snakes and birds of prey (Galán, 1988; Galán and Fernández, 1993), and eating many kinds of invertebrate groups (Carretero, 2004). Generally, these reptiles have highly diverse diets and such fact led to the belief that consumption was in accordance with the availability of prey in the environment (Avery, 1966; Pianka, 1986). However, more recent studies have evidenced that lacertids seem to be selective toward certain types and sizes of prey (Díaz and Carrascal, 1990; Dominguez and Salvador, 1990; Diaz, 1995). Selection seems to be dependent on several factors within a species, such as size and sex, as well as with seasonal climatic patterns. Seasonal variation one of the most important factors in Mediterranean areas, as there are strong fluctuations in climate, influencing aspects of lizard behavior, as well as availability of prey (Carretero, 2004).

Podarcis bocagei is a small, diurnal, insectivorous and highly sedentary lacertid (Galán, 1999). This species can be found in a variety of habitats, including agricultural fields and has a restricted distribution in the north-west of the Iberian Peninsula (Galán, 2009). Nevertheless, within this range it is widespread occupying a diversity of habitats from coastal dunes to mountains, including agroenviroments where is the commonest lizard species (Ribeiro *et al.*, 2010). *P. bocagei*'s diet is composed by many invertebrates, mostly arthropod groups (Pérez Mellado, 1982; Dominguez and Salvador, 1990; Diaz, 1995). Among commonly consumed groups are Coleoptera, Homoptera and Araneae, mainly prey that can be captured on the ground. Groups such as Araneae or Coleoptera are generally selected, while Formicidae are often rejected. A large variety of prey sizes are generally consumed, ranging from less than 1 mm to larger than 20 mm. Prey below 3 mm, such as Collembola or Thysanoptera are generally rejected, while larger sizes are positively selected at least in the Northwestern coast of Portugal (Dominguez and Salvador, 1990; Marques *et al.*, 2005; Marques and Carretero, 2006; Marques and Carretero, 2007).

Arthropods are extremely important animals in ecosystems and fulfill a large variety of ecological roles (Abbott *et al.*, 1979), being their roles in trophic webs noteworthy, as they serve as prey items for many vertebrates as well as invertebrates (Pearson and Derr, 1986; Vickery *et al.*, 2001). As the main source of food for lacertids, such as *P. bocagei*, arthropods assume a key role in these animals' habitat (Capinera, 2010). However, some *P. bocagei* populations inhabit agricultural areas (Galán, 2009; Kaliontzopoulou *et al.*, 2010), where agricultural practices are believed to impoverish biodiversity and where declines for many arthropod groups have been reported (Aebischer, 1991; Benton *et al.*, 2003; Sotherton and Holland, 2003). Pesticides are believed to be a major contributing factor for these declines (Aebischer, 1991) and in particular, herbicides which represent a large portion of the pesticides used worldwide (Conacher and Conacher, 1986). Herbicides are believed to affect not only the arable weed community but also the arthropod community in agroecosystems (Thomas and Marshall, 1999; Sotherton and Holland, 2003). Such effects on arthropods are believed to be mainly indirect as a result of changes in vegetation structure in cropped fields, as well as in field margins (Marshall and Moonen, 2002). Alteration of vegetation structure in margins can be important, because these structures serve as refuges, overwintering and reproduction sites as well as sources of food for vertebrates and invertebrates (Pollard *et al.*, 1974; Sotherton, 1984, 1985; Benton *et al.*, 2002; Marshall and Moonen, 2002; Sánchez-Bayo, 2010).

Hence, changes in arthropod communities may have impacts along trophic webs and can potentially affect insectivorous vertebrates (Southwood and Cross, 1969; Benton *et al.*, 2002; Wickramasinghe *et al.*, 2004; Simão *et al.*, under preparation), being, field margins an adequate field structure from which to sample prey items of lizards, because they support a diverse arthropod fauna (Sotherton, 1985). Besides, these structures are adjacent to walled field boundaries, which lizards use as habitat. Our objective was to assess availability of arthropods in field margins with distinct herbicide regimes and compare availability of prey items of *Podarcis bocagei* regarding taxonomic and size composition in two distinct seasons.

2 Materials and Methods

2.1 Prey items of *Podarcis bocagei*

Recognition of preferred lizard prey item groups was made using a recent study involving *Podarcis bocagei* (Marques *et al.*, 2005; Marques and Carretero, 2006). This study was used as a guideline, from which we retrieved information about prey items of this lacertid species. From this point onward the work of Marques and collaborators (2005; 2006) will be referred to as "reference study". The reference study was conducted in the localities of Mindelo and Vila Chã (41°18'N, 8°42'O), close to the study sites where pitfall traps were set (described in the section bellow), in absence of other lacertid species. Habitat was constituted by Atlantic dunes covered by psammophile vegetation with an annual average temperature of 12ºC and total annual precipitation of 1000-1200 mm. On a monthly basis throughout a year, lizards were caught and sacrificed, so that the complete stomach contents could be analyzed. Intestine contents were excluded to avoid bias in the results, because softer and easily digested preys are underrepresented according to Carretero and Llorente (2001). Considering spring and autumn, a total of 83 stomach contents were analyzed (5 were empty), 42 (2 empty) in autumn and 41(3 empty) in spring. Identification was carried out to the Order or Family level, separating larval from adult stages, with the use of a stereo microscope. Counting was made using cephalic capsules or wings and legs, following the minimum number criterion per sample. Organisms were measured with a millimetric scale partially bathed in alcohol and attached to a Petri dish. Individuals were grouped in classes from 0 to 20 in 1 mm intervals. All prey larger than 20 mm were assigned to class 20.

The entire arthropod community present in the above described sites was also sampled from the environment using a 1 m^3 biocenometer. Samples were collected to plastic bags, taken to the laboratory and kept in a freezer. The contents of the bags were emptied in trays filled with water to allow separation of individuals from the debris. Identification and measurement of individuals followed the procedure described above. This procedure was done to estimate the general patterns of trophic selection according to the availability of each arthropod taxa and class size. Results from the entire arthropod community in the environment were then compared with the results from stomach contents, to allow estimation of the trophic selection patterns by the lacertids. This procedure revealed which groups and prey lengths are most consumed, selected and rejected by this species (Marques *et al.*, 2005; Marques and Carretero, 2006).

Results and selection indices from the reference study were used as a guideline for pitfall trap results. Samples from all individuals were considered independently of sex and maturity. Seasons were considered separately.

2.2 Study sites and experimental design

Six geographically close sites with different herbicide regimes were selected. Fields were located in Northwestern Portugal in the municipalities of Vila do Conde (41°19'N, 8°40'W – exposed fields Exp 1, Exp 2, Exp 3 and Exp 4) and Vila Nova de Famalicão (41°26'N, 8°30'W – reference fields Ref 1 and Ref 2). Exposed fields had maize crops (*Zea mays* L.) in rotation with annual ryegrass (*Lolium multiflorum* Lam.), where a mixture of pesticides has been applied routinely over 30 years. The reference fields were represented by a pasture (not heavily grazed) and a farming field (several cultures) with no history of pesticide application. Annual mean temperature averages between 12.5-15 °C, total annual precipitation is 1400-1600 mm, whereas insolation is 2400-2500 h for exposed and 2300-2400 h for reference fields (Atlas do Ambiente, 1995). Soils are humic cambisols. Fields are bordered by a small vegetated margin of spontaneous grasses of variable width (from 20 cm to 1 m) and a stone wall, covered at some points with climbing plants. For the duration of our study, only herbicides were applied to exposed fields. The herbicides Spectrum™ (active ingredient (AI) – dimethenamid), Montana® (AI - glyphosate) and Controler T (AI - alachlor and terbuthylazine) were applied prior to the emergence of the crop, while Laddok Plus® (AI - dicamba and bentazon), Roundup® (AI - glyphosate) and Callisto® (AI - mesotrione), were applied after the emergence of the crop and weeds. In 2008 the herbicides applied were Spectrum™ in fields Exp 1, Exp 2 and Exp 4, Laddok Plus® in fields Exp 1 and Exp 4, Roundup® in field Exp 1, Controler T in field Exp 3 and Callisto® in field E3. In 2009 the following substances were applied: Laddok Plus® in fields E1 and E4, Montana® in field Exp 1 and Exp 4 and Callisto® in field Exp 3. A further description of the collection sites, including soil-pesticide profiles can be checked at (Amaral *et al.*, under preparation).

2.3 Pitfall sampling and processing

Arthropod sampling was carried out during 10 straight days in autumn (November 2008) and spring (April 2009). Non rainy days were selected when possible, to avoid biased results. Surface active arthropods were collected using pitfall traps (8 cm diameter). Ten traps were placed in each field close to the stone wall with a two meter spacing between them. Traps consisted of plastic containers dug into the soil, with the lip just a little bellow the ground surface. To prevent the entrance of small vertebrates, a 30 mm mesh wire piece was used and fixed with staples. Covers were positioned 20-30 mm above the trap to prevent flooding by rainwater. Traps were partially filled (1 to 2 cm) with a saturated salt solution to trap and preserve invertebrates through the collection period. After the arthropod sampling, traps were filled with ethanol (70%) and taken to the laboratory. Each sample was sieved using a 0.20 mm pore mesh and invertebrates sorted from the debris and preserved in a 70% ethanol solution until further analysis. Some of the pitfalls were not recovered from the fields, because of their destruction or filling with debris as a result of farmers work. This was the case of three pitfalls in autumn (fields Exp 2, Ref 1 and Ref 2) and two in spring (fields Exp 1 and Exp 2), leaving some fields with only nine samples.

Individuals were assigned to operational taxonomic units (OTU) (Sneath and Sokal, 1973) that in this case grouped individuals into Orders (with a few exceptions), separating larval from adult stages. Nomenclature and taxonomy of all groups was based on Barrientos (2004).

Body length was determined to the nearest 0.1 mm, being individuals measured from the anterior part of the head to the anus disregarding any appendages beyond. Individuals were grouped in 1 mm intervals, from class 0 to 20. Organisms smaller than 1 mm were assigned to class 0 and organisms larger than 20 mm were assigned to class 20. Individuals larger than 20 mm were grouped together because few individuals exceeded this length and to comply with methodology of Marques et al (2005; 2006) described above.

2.4 Statistical analysis

In the reference study, several descriptors were computed including percentage of presence (%P), percentage of numeric abundance (%N) and Use Index (IU). With use index of consumed prey (IUC) and use index of available prey (IUD), Ivlev's index (Ivlev, 1961) was computed using the modification by Jacobs (1974) (Marques *et al.*, 2005; Marques and Carretero, 2006). Confidence intervals of the Ivlev's index were calculated following Strauss (1979).

Pitfall data was pooled by field, so that each pitfall was considered as a replicate and seasons were analyzed separately. Kruskall-Wallis test was performed to determine whether abundance and size classes differed between fields. For significant differences, Dunn's *post-hoc* test was used to separate the distinct groups. For size classes only classes 1 to 11 were tested for differences, because of the very low catches in margins for bigger prey items. These analyses were performed using Statistica 7 software (StatSoft, 2004).

3 Results

3.1 Prey groups

Comparison between diet and availably of prey items in the reference study (Marques *et al.*, 2005; Marques and Carretero, 2006) revealed which prey items were consumed by *Podarcis bocagei*, as well as those selected or rejected according to their availability in the environment in both sampling seasons (Table 4). The prey group consumed varied less than availability in the environment between seasons. In spring, Coleoptera adults, Araneae and Isopoda were the most consumed prey items. Among the strongly selected were Lepidoptera, Coleoptera larvae, Coleoptera adults, Heteroptera, Hymenoptera adults and Araneae. Other groups, such as Collembola, Julidae and Pseudoscorpiones were strongly rejected. The group Formicidae was not consumed by the lizards, being completely rejected. In autumn, the most consumed prey were Araneae, Heteroptera, Coleoptera adults and Hymenoptera adults. The Lepidoptera larvae group was strongly selected, followed by Hymenoptera adults, Isopoda, Araneae and Pseudoscorpiones. Among the most strongly rejected groups were Julidae, Formicidae and Opiliones. Homoptera individuals were also rejected. The Heteroptera were eaten in accordance to their availability in the environment.

In the pitfall traps (see also Simão *et al.*, under preparation), a total of eight prey groups were captured in spring, namely Coleoptera adults, Araneae, Isopoda, Coleoptera larvae, Homoptera, Heteroptera, Hymenoptera adults (excluding ants) and Lepidoptera larvae. Some of the rejected groups were also found in our fields in high amounts, namely Formicidae, Julidae and Collembola. Total number of prey item catches was 2141 individuals, with Coleoptera being the most and Lepidoptera larvae the least captured prey item. In autumn, nine prey groups were captured in pitfalls, namely Araneae, Heteroptera, Coleoptera adults, Hymenoptera adults (excluding ants), Lepidoptera larvae, Isopoda, Coleoptera larvae, Pseudoescorpiones and Homoptera. Some of the rejected preys were also found, namely Formicidae, Opiliones and Julidae. Total number of prey item catches was 1173, being the most captured prey items Araneae and Coleoptera adults and the least captured Heteroptera.

Table 4 – List of the consumed, selected and rejected prey items by *P. bocagei* in Mindelo in both sampling seasons (Marques *et al.*, 2005; Marques and Carretero, 2006). Prey items are arranged from most to least consumed based on resource use index (IUC). Selection or rejection of prey items according to availability in the environment is also presented based on Ivlev index: positive values represent a positive selection and negative values evidence a rejection toward the specific prey items. Only prey items in common with pitfall trap catches are represented in the table, all others are represented together as "others". Represented with permission.

Spring	Consumed (IUC)	Ivlev index
Coleoptera (imago)	45.8	0.9
Araneae	15.3	0.6
Isopoda	11.0	0.4
Coleptera (larva)	5.7	0.9
Homoptera	5.7	0.5
Heteroptera	3.6	0.8
Hymenoptera (imago)	3.2	0.7
Lepidoptera (larva)	2.2	1.0
Collembola	0.0	-1.0
Julidae	0.0	-1.0
Pseudoescorpiones	0.0	-1.0
Formicidae	0.0	-1.0
Others	7.5	–
Total	100	–
Autumn	**Consumed (IUC)**	**Ivlev index**
Araneae	23.1	0.7
Heteroptera	19.2	0.0
Coleptera (imago)	10.8	0.5
Hymenoptera (imago)	10.2	0.9
Lepidoptera (larva)	6.4	1.0
Isopoda	2.6	0.8
Coleptera (larva)	2.6	0.4
Pseudoescorpiones	2.4	0.3
Homoptera	0.8	-0.6
Julidae	0.0	-1.0
Opiliones	0.0	-1.0
Formicidae	0.0	-1.0
Others	21.9	–
Total	100	–

In spring, differences were found in catches between fields, except for Lepidoptera larvae (Table 5). Also, field Exp 1 contained the highest numbers of five prey groups and total prey groups, although catches were not always significantly higher than those of all other fields. Generally reference fields had low prey numbers, although not always significantly lower (Figure 13). In autumn, only six of the prey groups exhibited significant differences between fields (Araneae, Isopoda, Coleoptera larva, Homoptera, Hymenoptera adults and Pseudoscorpiones) (Table 5). Also, field Ref 2 had higher abundances for Araneae, Isopoda and Pseudoescorpiones, Exp 1 exhibited higher numbers of Coleoptera larvae and Exp 3 had higher catches of one OTU for Hymenoptera adults excluding Formicidae (Figure 13).

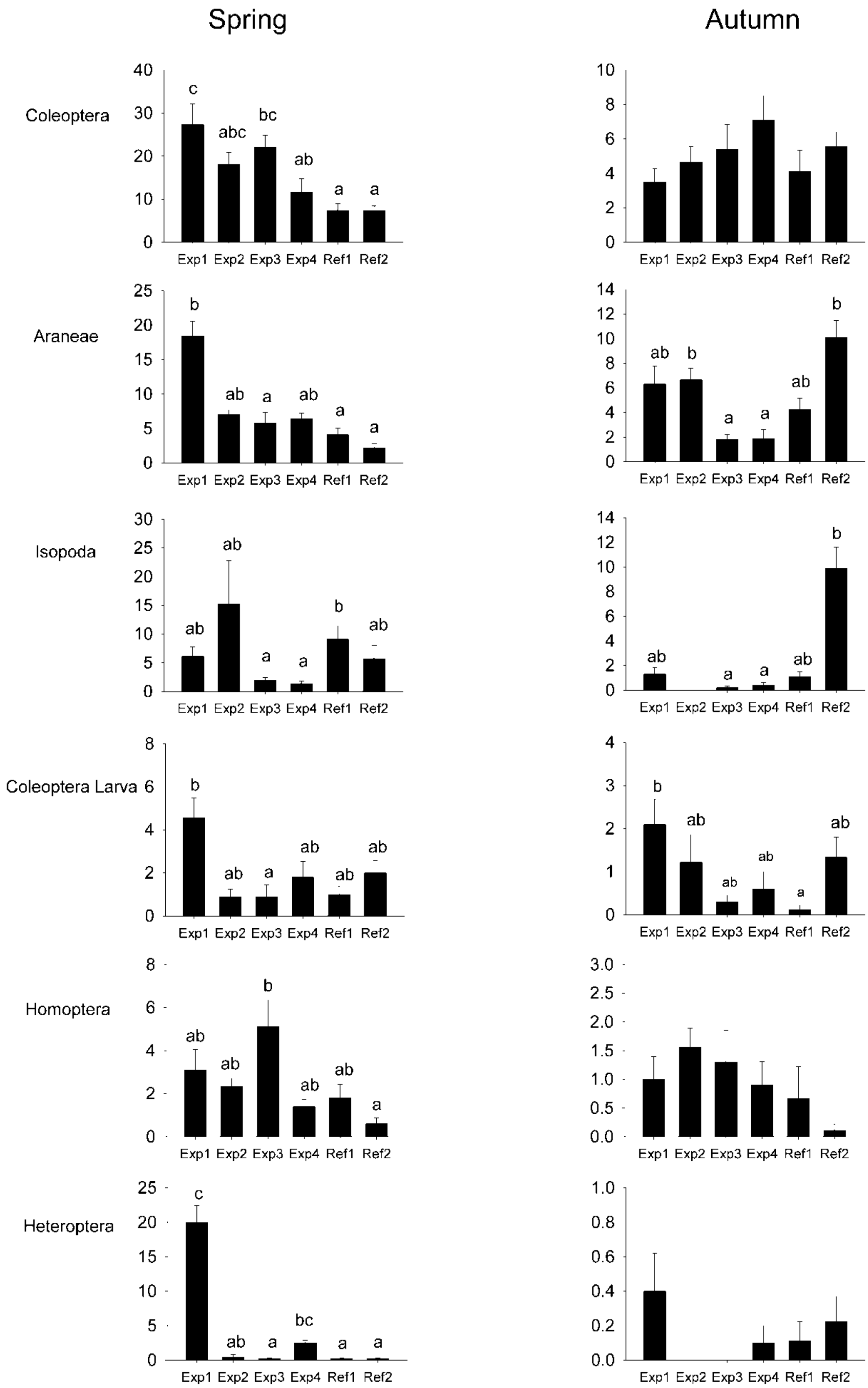

Figure 13 – OTU catches in spring and autumn. Values are mean catches per field. Columns with the same letter are not statically distinct. Exposed fields: Exp 1, Exp 2, Exp 3 and Exp 4; reference fields: Ref 1 and Ref 2. Hymenoptera catches exclude Formicidae. Coleoptera and Hymenoptera do not include larvae.

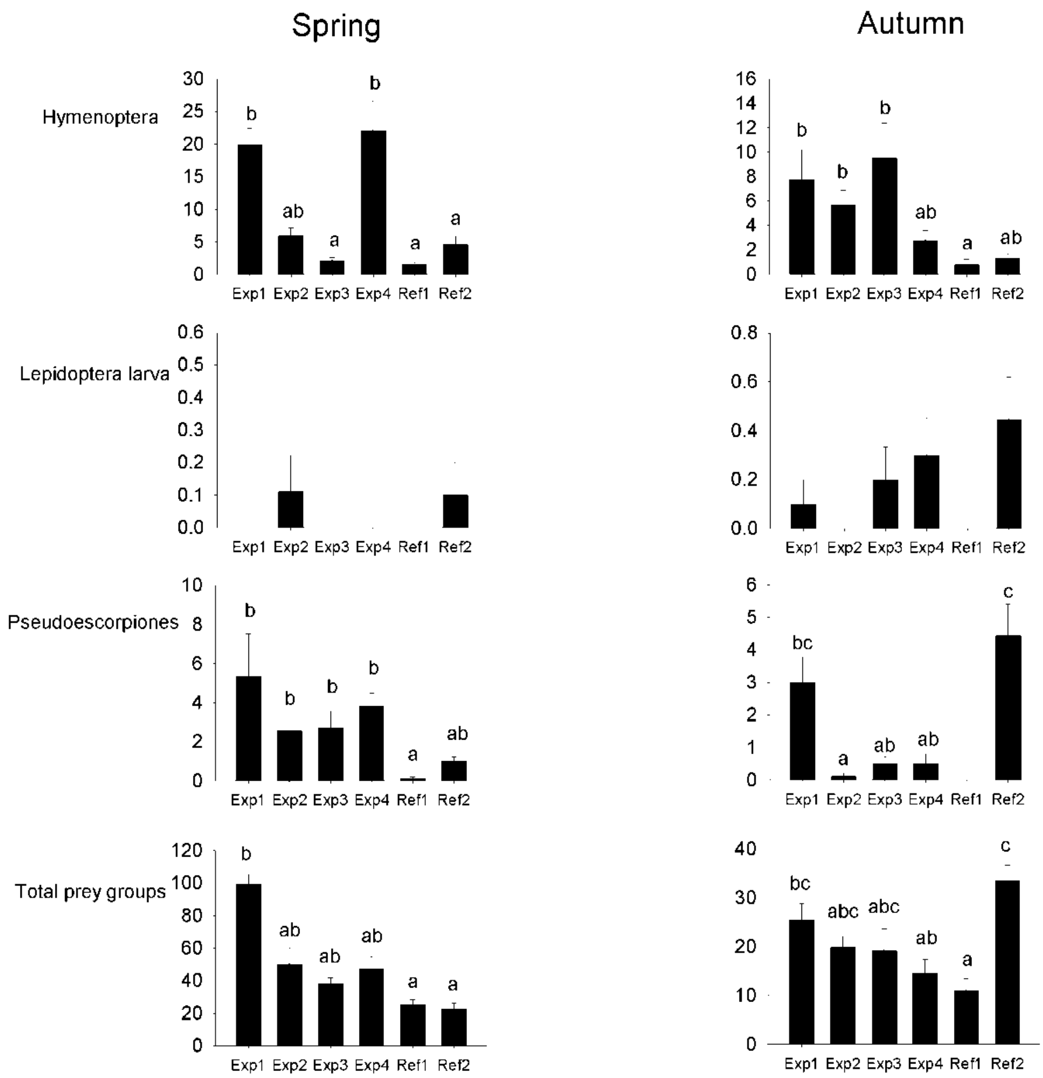

Figure 13 – Continued.

Table 5 – Results of the Kruskal-Wallis test (H) for all prey groups in spring and autumn.

	Spring		Autumn	
	H	p	H	p
Coleoptera (imago)	30.376	< 0.001	5.352	0.375
Araneae	32.890	< 0.001	30.547	< 0.001
Isopoda	17.879	0.031	33.987	< 0.001
Coleptera (larva)	16.076	0.007	15.187	0.010
Homoptera	17.865	0.031	12.712	0.026
Heteroptera	43.871	< 0.001	6.514	0.259
Hymenoptera (imago)	42.465	< 0.001	20.593	0.001
Lepidoptera (larva)	4.196	0.522	9.667	0.085
Pseudoescorpiones	28.506	< 0.001	37.142	< 0.001
Total prey groups	32.416	< 0.001	21.988	< 0.001

3.2 Size analysis

The reference study by Marques et al (2005; 2006) evidenced a size consumption by *P. bocagei* with a distinct pattern between sampling seasons. Practically all size classes were consumed on both sampling seasons but not in the same amounts. Classes 2 to 8 were usually the most consumed, with size six being preferred in spring and size class three in autumn. However, selection patterns were not similar. In spring, size classes from zero to two were rejected, as well as some larger size classes. Classes four to eight were positively selected and classes three, ten, 12 and 14 were eaten in accordance to availability in the environment. In autumn a large part of the size classes were rejected, excluding five, seven, eight, 14, 15 and 16, which were positively selected (Table 6).

Table 6 – Size classes selected, eaten according to their presence in the environment (PE) and rejected, for both sampling seasons in the reference study (Marques *et al.*, 2005; Marques and Carretero, 2006). Represented with permission.

	Spring	Autumn
Size classes	Ivlev index	Ivlex index
0	-1	-1
1	-0.8	-0.9
2	-0.7	-0.5
3	0.3	0.2
4	-0.2	0.1
5	-0.3	0.2
6	0.8	0.7
7	0.3	0.2
8	0.9	0.3
9	-1	-0.5
10	-0.1	0
11	-1	-0.6
12	-1	-1
13	-0.6	-1
14	0	-1
15	-0.1	0
16	-1	-1
17	–	1
18	-1	–
20	-0.7	-0.7

*Some classes are not represented because they were not found in either the stomach contents or the biocenometers in the reference study

Pitfall results evidenced that in spring the most captured size was class one for all fields and in autumn the most captured class varied between fields (Table 7). Regarding spring, significant differences were found between fields for all most consumed class sizes by *Podarcis bocagei* (two to eight). In spring, field Exp 1 had the highest catches for all size classes, whilst field Ref 2 catches were always among the lowest for these size classes. For the most consumed class in this season, field Exp 1 also had the highest catches. In autumn, classes two and six did not evidence significant differences between fields, while for the other most consumed classes significant differences were found. Field Ref 2 evidenced the highest catches for classes seven and eight and for class three, the most consumed in this season. Field Exp 2 evidenced the highest catches for classes 4 and 5 (Table 8).

Table 7 – Total number of prey items found in pitfall traps for consumed OTU in each of the size classes in spring and in autumn for each of the fields.

	Spring						Autumn					
Size class	Exp 1	Exp 2	Exp 3	Exp 4	Ref 1	Ref 2	Exp 1	Exp 2	Exp 3	Exp 4	Ref 1	Ref 2
0	45	13	24	24	4	0	59	28	69	16	0	10
1	231	140	141	259	38	70	80	30	48	29	7	59
2	116	139	128	40	18	42	31	25	46	53	31	32
3	99	70	33	47	22	31	25	13	6	13	21	44
4	44	35	23	36	22	15	15	34	5	12	12	21
5	87	22	14	23	24	11	13	34	3	7	15	9
6	93	7	9	11	24	11	7	5	5	5	0	8
7	102	8	10	22	34	5	6	0	3	2	3	20
8	54	7	6	15	15	8	1	0	1	0	4	24
9	24	6	6	9	18	6	7	2	1	1	2	28
10	6	4	9	5	13	12	5	4	0	1	0	14
11	13	4	2	3	6	2	2	1	1	3	3	10
12	6	5	3	3	4	7	0	2	0	1	1	1
13	4	6	0	1	2	2	1	0	1	0	0	3
14	3	4	0	1	2	1	0	0	0	0	1	2
15	7	1	0	5	3	2	0	1	0	0	0	1
16	2	2	0	0	1	4	0	0	0	0	0	4
17	0	1	0	2	0	2	1	0	0	0	0	4
18	5	0	0	0	0	1	0	0	1	1	0	0
19	0	0	0	0	0	2	0	0	1	0	0	3
20	2	0	0	4	2	3	1	0	1	2	0	3

Table 8 – Differences between size classes of each field in spring and autumn using Kruskal-Wallis test (H) and Dunn's *post-hoc* test for multiple comparisons. Distinct letters between fields indicate statistical differences.

Size class	Spring							
	Exp 1	Exp 2	Exp 3	Exp 4	Ref 1	Ref 2	H	p
0	c	abc	bc	abc	ab	a	24.954	0.001
1	b	ab	ab	b	a	a	35.734	<0.001
2	d	bcd	cd	abc	a	ab	33.837	<0.001
3	c	bc	ab	abc	a	ab	30.244	<0.001
4	b	ab	ab	ab	ab	a	15.101	0.010
5	b	ab	a	a	a	a	28.551	<0.001
6	b	a	a	a	ab	a	24.981	0.001
7	b	a	a	ab	ab	a	33.649	<0.001
8	b	a	a	ab	ab	a	27.041	0.001
9							16.667	0.005
10							8.667	0.123
11							9.265	0.099
	Autumn							
0	Exp 1	Exp 2	Exp 3	Exp 4	Ref 1	Ref 2	H	p
1	b	b	b	ab	a	ab	22.546	<0.001
2	b	ab	ab	ab	a	b	25.477	0.001
3							6.587	0.253
4	ab	ab	a	a	ab	b	20.065	0.001
5	ab	b	a	ab	ab	ab	17.818	0.003
6	ab	b	a	a	ab	a	23.170	<0.001
7							6.074	0.299
8	ab	a	ab	a	ab	b	22.416	<0.001
9	a	a	a	a	ab	b	38.586	<0.001
10	ab	a	a	a	a	b	25.227	0.001
11							21.995	<0.001

4 Discussion

As the main source of *P. bocagei*'s diet, arthropods represent a key factor in this lizard's habitat. However, factors that reduce this resource could potentially have harmful effects on lizard populations. In this study, we assessed availability of consumed prey groups by *P. bocagei*. Our results indicate that availability of prey items varied with season, with spring evidencing higher numbers for all prey groups, except for Lepidoptera larvae. Differences between fields also varied with season, with some of the fields evidencing higher catches in one of the seasons but not in the other. Differences related with exposure to herbicides were not consistent and no clear pattern of variation between herbicide treated and non-treated fields was detected. Despite this inconsistency, some fields evidenced higher catches for many of the main prey groups, as well as for preferred prey sizes. In spring, field Exp 1 evidenced higher catches for many prey groups including the most consumed prey and the most consumed sizes in this season. In autumn, field Ref 2 evidenced higher availability for many prey groups, including the most consumed group and size, although differences between fields were less marked than in spring.

P. bocagei is a ground-dwelling lizard that actively searches for prey (Galán, 2009). Therefore prey is expected to be mainly consumed on the ground or surface of the walls. In this situation, pitfalls provide an adequate method for estimating prey availability for these reptiles, because they sample mainly epigeic arthropods. Although this method has some important bias, such as the dependence of captures on species activity (Greenslade, 1964; Topping and Sunderland, 1992), it is also a low time and cost demanding method and hence easily replicable when compared to other methods. The reference study used a distinct sampling method; nevertheless results were only used to determine the selection patterns of the distinct groups and sizes according to their differential availability and not to fixed values. According to the reference study (Marques *et al.*, 2005; Marques and Carretero, 2006), the main prey was selected following a nutrient optimization pattern, which assumes that prey are distinct in nutritional content and therefore predators choose them in accordance with their nutritional requirements (Stamps *et al.*, 1981). Since the reference study was made in the same region as the pitfall survey, we can assume that the nutritional needs will be similar for these animals. Prey availability however, will in theory be distinct since the reference study was made in the dune ecosystem, while the pitfall survey took place in agricultural ecosystems. Consequently, the selection indexes may not be the same, as lizards would be expected to select roughly the same groups to suffice their nutritional needs, but availability of prey groups would not be the same. Between fields with distinct arthropod communities, it would also be expected for lizards to select prey in a different way. Our pitfall results evidence that the communities of fields are distinct, which implies that selection of prey items by *P. bocagei* may be distinct between fields, even if proportion of consumed groups does not vary much.

The major factor expected to affect catches was the application of herbicides and we expected exposed fields to have lower arthropod catches. However, results were not consistent in terms of herbicide application and exposed fields did not exhibit lower catches than reference fields (see also Simão *et al.*, under preparation). Theoretically, herbicides affect plant communities of margins, making them poorer in term of diversity and cover (e.g. de Snoo, 1999; Taylor *et al.*, 2006). Therefore, arthropod communities were expected to have lesser amounts of food and shelter (Pollard *et al.*, 1974; Sotherton, 1984), as well as potentially being directly affected by herbicides (e.g. Salminen *et al.*, 1997). These factors together could have impacts on arthropod catches, as several studies have shown (e.g. Moreby and Southway, 1999; Thomas and Marshall, 1999). Nevertheless, this absence in herbicide use pattern may indicate that other factors are blurring the results. Several possibly influencing factors were not assessed, such as plant cover and diversity, as well as landscape structure (Hunter, 2002).

Despite herbicide application alone not explaining the patterns found, actual differences existed between fields. Such differences in availability of arthropod groups and most consumed sizes may have implications for *P. bocagei* sub-populations. A substantial amount of the studies regarding food items of vertebrates in agroecosystems focus on important arthropods for birds. Availability of bird prey items in crops and field margins have been shown to be affected by herbicides, with reduced prey availability potentially having impacts on bird populations, especially when the more vulnerable juveniles are affected (e.g. Southwood and Cross, 1969; Moreby and Southway, 1999; Benton *et al.*, 2002; Taylor *et al.*, 2006). However, small endothermic vertebrates with high metabolic rates and energy requirements, require much time for foraging activities (Díaz and Carrascal, 1993), while the energy requirements of lacertids are much lower than endothermic animals of similar sizes (Pough, 1980) and comparisons between such distinct organisms must be done carefully. Nevertheless, studies regarding lizards have assumed or implied that food scarcity has implications for individuals such as alteration of the reproductive success, growth and survival rates and size at maturity (Ballinger, 1977; Stamps and Tanaka, 1981). Studies concerning lacertids have found evidence of alteration in growth rates (Iraeta *et al.*, 2006) and population size (Díaz and Carrascal, 1991) of animals in populations with reduced amounts of food. Despite this, agricultural impacts on reptile communities vary, with some species being negatively affected (Glor *et al.*, 2001), while others are positively affected (Biaggini *et al.*, 2009). The lacertid species *P. bocagei* is extremely euryphagous and consumes a large variety of arthropod groups. Therefore it is expected to be adaptable to variations in arthropod communities resulting from agricultural practices. This fact could explain why this species (Ribeiro *et al.*, 2010) and others of the same genus (Graziani *et al.*, 2006; Paggetti *et al.*, 2006; Biaggini *et al.*, 2009) survive in agroenvironments were other more sensitive reptiles disappear.

In conclusion, herbicide treatment did not explain differences between availability of prey items of *Podarcis bocagei* and other unassessed factors could be obscuring our results. Nevertheless, differences between fields were found in availability of prey items in both seasons. Results indicate that fields are distinct in availability of prey and most consumed sizes and some of the fields may be more favorable to *P. bocagei* individuals. Despite this, the extremely euryphagous diet of this species may be the reason why these animals survive in agroenvironments, while other more sensitive reptiles disappear. Further work in determining the consumed prey by *P. bocagei* in these fields and assessment of plant cover and diversity, as well as landscape structure would be of value.

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Chapter IV – Discussion and Conclusion

Discussion and Conclusion

Different herbicide regimes can theoretically affect the biodiversity present in field margins. This may occur in distinct ways, because these substances can affect organisms directly, as well as vegetation structure and composition and soil physical conditions, thus altering habitat conditions (Pollard *et al.*, 1974; Sotherton, 1985; Marshall and Moonen, 2002). Arthropod communities can potentially be affected and alterations in these communities may have impacts on higher trophic levels as well as on ecosystem functioning by affecting functional groups (Swift *et al.*, 1996; Taylor *et al.*, 2006; Clough *et al.*, 2007). *Podarcis bocagei*, as an arthropod feeding predator commonly found in agroecosystems (Marques and Carretero, 2006; Galán, 2009), may also be affected by these changes.

In general, no clear relationship between herbicide application and a reduction in biodiversity was observed when herbicide treated fields were compared with non treated fields. Surprisingly, herbicide treated margins had higher activity-density and group richness than reference margins in spring. In autumn, differences were less marked, with a single reference field exhibiting the highest arthropod activity-density and group richness. Furthermore, reference fields did not seem very similar in arthropod community composition and were actually more similar to some of the exposed fields than to one another.

None of the arthropod guilds seemed to be negatively affected by the herbicide treatment despite the significant differences found between fields; in fact predators presented higher activity-density and richness in all exposed fields when compared to reference fields in spring. Since exposed margins evidenced higher numbers for all guilds, these higher predator numbers may be explained by the potential higher number of prey. In autumn the differences in guilds were less marked and none of the guilds exhibited differences between treatments.

Seasonal differences (spring vs. autumn) were very important both in terms of arthropod numbers and in the types of groups found, because spring samples exhibited higher number of arthropods, as well as higher number of groups. Also, in spring, catches were dominated by the Formicidae, while in autumn, catches were more evenly distributed among groups. Additionally, some groups were exclusive of a particular season, such as Mymaridae and Nemesidae which were exclusive of autumn samples and Geotrupidae and Scarabaeidae that were exclusive of spring samples. Other groups such as Entomobryomorpha and Porcellionidae were relatively abundant in both seasons. Differences found between seasons may be related to climatic variations in temperature and precipitation, as well as differences in the life cycles of the distinct arthropod groups.

Availability of prey items of *Podarcis bocagei* in each of the fields was found to be distinct. However, herbicide effects were not enough to explain the differences found between fields. Although fields differed in the number of arthropod groups found, results were not consistent between seasons, being some fields favorable to these lizards in one season but not in the other. Prey size analysis followed the same pattern. These results indicate that some of the fields may be more favorable than others to lizard communities, at least at some points of the year. Nevertheless, *Podarcis bocagei* was actually found in all of the studied fields, which seems to point that all of the fields support arthropod communities that are sufficient to sustain these lizard communities. The fact that this species preys on a large variety of arthropod groups may explain why these animals are able to survive in agroecosystems, while other reptile species are not.

Differences found between fields for all these parameters were not easily explained based only in herbicide usage. This fact indicates that other factors may be obscuring the results. Vegetation structure and composition, as well as landscape structure were not assessed, but are factors that may be related to the differences found between fields. Assessment of these factors could be useful to shed some light in the source of the differences found.

Further work

Further work in this field should consider several research topics:

- Determine in laboratory studies if the herbicides and herbicide combination used in the present study have any direct effects on soil arthropods at the recommended application rates. This would provide a basis to determine if negative effects, both lethal and non lethal, could be caused by these substances or if effects on soil arthropods would only be indirect;
- In the study fields, assess the floral composition of the field margins in every season and compare with new data from pitfall traps and other sampling methods specific for flying arthropods and flying insects;
- Assess the diet of *Podarcis bocagei* individuals living in each of the fields in order to have a comparison basis with availability of prey groups;
- Assess the landscape and habitat structure around the fields, so that it could be compared between fields and determine if there are significant differences.

These topics would allow for more specific results, which would provide a more precise data set that could more easily explain differences between the fields, in terms of abundance, species richness, guild composition and *Podargis bocagei* prey items.

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Appendix A – List of groups and corresponding guilds

Class Arachnida

Order Araneae	
Family Agelenidae	Predators
Family Clubionidae	Predators
Family Dysderidae	Predators
Family Gnaphosidae	Predators
Family Linyphiidae	Predators
Family Liocranidae	Predators
Family Lycosidae	Predators
Family Nemesidae	Predators
Family Oonopidae	Predators
Family Salticidae	Predators
Family Tetragnathidae	Predators
Family Zodariidae	Predators
Order Opiliones	
Family Nemastomatidae	Predators
Family Phalangiidae	Predators
Family Sclerosomatidae	Predators
Family Trogulidae	Predators
Order Pseudoescorpiones	
Family Chthoniidae	Predators
Family Garypidae	Predators
Family Neobisiidae	Predators

Class Chilopoda

Order Geophilomorpha	Predators
Order Lithobiomorpha	
Family Lithobiidae	Predators
Order Scolopendromorpha	

Family Cryptopidae	Predators
Order Scutigeromorpha	
Family Scutigeridae	Predators
Class Collembola	
Order Entomobryomorpha	Saprophagous and fungal feeders
Order Poduromorpha	Saprophagous and fungal feeders
Order Symphypleona	Saprophagous and fungal feeders
Class Diplopoda	
Order Craspedosomatida	
Family Craspedosomatidae	Saprophagous and fungal feeders
Order Glomerida	
Family Glomeridae	Saprophagous and fungal feeders
Order Julida	
Family Julidae	Saprophagous and fungal feeders
Order Polydesmida	
Family Polydesmidae	Saprophagous and fungal feeders
Order Polyxenida	Saprophagous and fungal feeders
Class Euentomata	
Order Coleoptera	
A larva	N/A
Family Anthicidae	Saprophagous and fungal feeders
Family Apionidae	Herbivores
B larva	N/A
Family Byrrhidae	Saprophagous and fungal feeders
C larva	N/A
Family Cantharidae	Predators
Family Carabidae	Predators
Family Chrysomelidae	Herbivores
Family Cicindelidae	Predators
Family Cleridae	Predators

Family Coccinellidae	Predators
Coccinellidae larva	Predators
Family Colydiidae	Predators
Family Corylophidae	Saprophagous and fungal feeders
Family Cryptophagidae	Saprophagous and fungal feeders
Family Curculionidae	Herbivores
D larva	N/A
Family Dermestidae larva	Saprophagous and fungal feeders
Family Dryopidae	Herbivores
E larva	N/A
Family Elateridae	Herbivores
Family Erotylidae	Saprophagous and fungal feeders
F larva	N/A
G larva	N/A
Family Geotrupidae	Saprophagous and fungal feeders
H larva	N/A
Family Histeridae	Predators
Family Hydrophilidae	Saprophagous and fungal feeders
I larva	N/A
Family Lampyridae	Predators
Lampyridae larva	Predators
Family Lathridiidae	Saprophagous and fungal feeders
Family Leiodidae	Saprophagous and fungal feeders
Family Melyridae	Predators
Family Nitidulidae	Saprophagous and fungal feeders
Family Ptiliidae	Saprophagous and fungal feeders
Family Ptinidae	Saprophagous and fungal feeders
Family Scarabaeidae	Saprophagous and fungal feeders
Family Scydmaenidae	Predators
Family Silphidae	Saprophagous and fungal feeders

Silphidae larva	Saprophagous and fungal feeders
Family Sphindidae	Saprophagous and fungal feeders
Family Staphylinidae	Predators
Family Tenebrionidae	Saprophagous and fungal feeders
Family Throscidae	Saprophagous and fungal feeders
Order Dermaptera	
Family Forficulidae	Predators
Order Diplura	
Family Campodeidae	Herbivores
Order Diptera	
Family Calliphoridae	Saprophagous and fungal feeders
Family Camillidae	Saprophagous and fungal feeders
Family Cecidomyiidae	Herbivores
Family Ceratopogonidae	Predators
Family Chironomidae	N/A
Chironomidae larva	Saprophagous and fungal feeders
Family Chloropidae	Saprophagous and fungal feeders
Family Diastatidae	Saprophagous and fungal feeders
Family Drosophilidae	Saprophagous and fungal feeders
Family Fanniidae	Saprophagous and fungal feeders
Family Hybotidae	Predators
Family Limoniidae	Saprophagous and fungal feeders
M larva	N/A
Family Muscidae	Saprophagous and fungal feeders
Family Odiniidae	Saprophagous and fungal feeders
Family Opomyzidae	Herbivores
Family Pallopteridae	Saprophagous and fungal feeders
Family Phoridae	Herbivores
Family Psychodidae	Saprophagous and fungal feeders
Family Scathophagidae	Predators

Family Sciaridae	Saprophagous and fungal feeders
Sciaridae/Mycetophilidae larva	Saprophagous and fungal feeders
Family Sepsidae	Herbivores
Sepsidae larva	Saprophagous and fungal feeders
Family Sphaeroceridae	Saprophagous and fungal feeders
Family Syrphidae	Predators
Family Tachinidae	Saprophagous and fungal feeders
Family Tipulidae	Saprophagous and fungal feeders
Tipulidae larva	Saprophagous and fungal feeders
Family Xylomiidae larva	Saprophagous and fungal feeders
Order Hemiptera	
Family Aphididae	Herbivores
Family Cicadellidae	Herbivores
Cicadellidae ninfa	Herbivores
Family Cimicidae	N/A
Family Cydnidae	Herbivores
Family Lygaeidae	Herbivores
Lygaeidae ninfa	Herbivores
Family Reduviidae	Predators
Family Tingidae	Herbivores
Order Hymenoptera	
Family Aphelinidae	Parasitoids and parasites
Family Apidae	Herbivores
Family Brachonidae	Parasitoids and parasites
Family Ceraphronidae	Parasitoids and parasites
Family Diapriidae	Parasitoids and parasites
Family Eulophidae	Parasitoids and parasites
Family Figitidae	Parasitoids and parasites
Family Formicidae	Ants
Formicidae larva	N/A

Family Ichneumonidae	Parasitoids and parasites
Family Megaspilidae	Parasitoids and parasites
Family Mymaridae	Parasitoids and parasites
Family Platygasteridae	Parasitoids and parasites
Family Proctotrupidae	Parasitoids and parasites
Family Pteromalidae	Parasitoids and parasites
Family Scelionidae	Parasitoids and parasites
Order Isoptera	
Family Rhinotermitidae	Herbivores
Order Lepidoptera	
Family Arctiidae larva	Herbivores
Family Geometridae larva	Herbivores
X larva	Herbivores
Y larva	Herbivores
Z larva	Herbivores
Order Mecoptera larva	Saprophagous and fungal feeders
Order Microcoryphia	
Family Machilidae	Herbivores
Order Orthoptera	
Family Acrididae	Herbivores
Family Gryllidae	Herbivores
Order Siphonaptera	Parasitoids and parasites
Order Thysanoptera	
Family Merothripidae	Herbivores
Family Phlaeothripidae	Herbivores
Order Trichoptera	
Family Limnephilidae larva	Herbivores
Class Malacostraca	
Order Isopoda	
Family Armadilidiidae	Herbivores

Family Porcellionidae	Saprophagous and fungal feeders
Class Symphyla	
Order Symphyla	
Family Scolopendrellidae	N/A
Family Scutigerellidae	Herbivores