INTRAGUILD PREDATION AMONG GENERALIST PREDATORS IN WINTER WHEAT

JEFFREY STEWART DAVEY

A thesis submitted to Cardiff University for the higher degree of Doctor of Philosophy

September 2010

Cardiff School of Biosciences
Cardiff University

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Form: PGR_Submission_200701

NOTICE OF SUBMISSION OF THESIS FORM: POSTGRADUATE RESEARCH

APPENDIX 1:
Specimen layout for Thesis Summary and Declaration/Statements page to be included in a Thesis

DECLARATION

This work has not previously been accepted in substance for any degree and is not concurrently submitted in candidature for any degree.

Signed ..... (candidature) Date 18/2/11.....

STATEMENT 1

This thesis is being submitted in partial fulfilment of the requirements for the degree of .....(insert MCh, MD, MPhil, PhD etc, as appropriate)

Signed ..... (candidature) Date 18/2/11.....

STATEMENT 2

This thesis is the result of my own independent work/investigation, except where otherwise stated.
Other sources are acknowledged by explicit references.

Signed ..... (candidature) Date 18/2/11.....

STATEMENT 3

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

Signed ..... (candidature) Date 18/2/11.....

STATEMENT 4: PREVIOUSLY APPROVED BAR ON ACCESS

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loans **after expiry of a bar on access previously approved by the Graduate Development Committee.**

Signed ..... (candidature) Date 18/2/11.....

Abstract

Modern annual arable crops are susceptible to outbreaks of pests due mainly to the uniform habitats that agricultural fields represent. Cereals are particularly prone to infestations of aphids, which may damage the crop directly through herbivory, or indirectly by acting as vectors of disease. These insects, however, have a large range of invertebrate natural enemies, which under certain circumstances, can maintain infestations below economically damaging levels. Greater habitat complexity at landscape and farm scale usually leads to more diverse assemblages of natural enemies at the field scale, but such diversity less often translates to a higher risk for pests. When higher natural enemy diversity is associated with lower levels of pest control, intraguild predation (IGP) is often cited as one of the primary antagonsitic mechanisms. IGP occurs where predators not only compete for the same resource, but also partake in a trophic interaction with one another. Controlled experiments suggest that the niche proximity of predators relative to each other and their shared prey may help predict the outcome of multiple-predator interactions.

The primary aim of this thesis was to assess levels of IGP amongst generalist invertebrate predators and to elucidate their spatial patterns, in fields of winter wheat (*Triticum aestivum*), an important cereal crop in North-West Europe. The chief objective was to establish the regulatory abilities of these predators in the control of aphids. Post-mortem gut content analysis using PCR was used to establish the intensity of IGP by two polyphagous predators, the carabid beetles *Pterostichus melanrius* and *P. madidus*, on a number of insectivorous linyphiid spiders and their shared aphid prey. Each of the spiders tested was found to suffer IGP. Predation rates were adjusted using data from controlled feeding trials and resampled using Monte Carlo models to test the hypothesis that predation was density-dependent. In one experiment, the web-occupying linyphiid *Tenuiphantes tenuis* was consumed by up to a third of *P. melanarius*. Predation rates by carabids on the linyphiid *Bathyphantes gracilis* were consistently lower than expected. *B. gracilis* also relies principally on its web to capture prey, but builds these webs significantly higher in the wheat stem than *T. tenuis*. Preferences for intraguild

prey species more likely to hunt aphids on the ground, the tetragnathid spider *Pachygnatha degeeri*, and the linyphiids *Erigone* spp. (*E. atra* and *E. dentipalpis*), were less consistent than those species more dependent on their webs to hunt aphids and other prey. While many factors may contribute to the outcomes of multi-predator interactions, these findings broadly supported the hypothesis that niche proximity of intraguild predators is positively related to levels of disruption due to intraguild interactions.

Acknowledgements

The work in this thesis would not have been possible without the help of a long list of people. To name but a few...

- Profs Bill Symondson and Mike Bruford (supervisors) and Dr John Holland (CASE partner, Game and Wildlife Conservation Trust), who made the whole thing possible.
- Staff at Rothamsted who collected the samples used in Chapters 3, 4 and 5, especially Drs James Bell (for copious advice and ideas and obscure spider papers) and Dave Bohan.
- Staff, students and affiliates, past and present, at Cardiff University, particularly Drs Ian Vaughan (statistical advice and coding and development of Monte Carlo models), Andy King (lab guru, DNA extractions of Rothamsted carabids), Jo Cable (advice and internal examiner), Peter Ferns, Rob Thomas and Mark Jervis (for allowing me to escape to Skomer for a week). Also members of the G10/C5.15 lab, including Dave, Renata, Rob, Ben, Alex, Milena, Geoff, Adam, Ciara, Catherine, Rhys, Vicky, the Jo's, the Mafaldas, Rui, Tania, Dan, Bjorn, Tim, Rafa, Mireille, Gabi, Muya, Nia for counting lots of collembola, and many more whose names escape me (names often escape me!) for tolerating my cooking, groaning and occasional diatribes.
- Staff at Sheffield for their assistance with the screening on ABI sequencer, especially Andy Krupa and Deb Dawson.
- Keith Sunderland, whose bibliography of spider research was invaluable.
- Robert Reader of Burdon's Farm, Wenvoe and Peter Davies of Slade Farm, Southerndown for allowing me to sample from their fields. The staff of the field centre in Southerndown also kindly agreed to let me use their facilities during fieldwork.
- My friends outside the university and my family, who have always been extremely understanding and supportive, not least my wife Viola, who helped with field sampling, gave me invaluable advice on stats, proofread just about my whole thesis and is simply wonderful. I love her very much.
- My Dad, who never failed to indulge my curiosity.

Table of Contents

Chapter 1: Introduction

Chapter 2: Methods and preliminary experiments

Figures and Tables

Chapter 2

Chapter 3

Chapter 4

Chapter 5

1. Introduction

1.1. Arable food production past and present

Populations of humans, like any other species, are ultimately limited by their food resources. Intensification of food production arguably began with the first domestication of animals and plants in South-West Asia circa 11,000 years ago (Zohary & Hopf 1993). Such domestication allowed populations of humans to become sedentary and therefore grow more quickly as their food supply became more reliable (Diamond 2000). Since this time, rising population growth has lead to ever more demands on agriculture, which has tended to both intensify and use more land, resulting in increased food production which in turn allows further population growth (Diamond 2000). A milestone in the satisfaction of these demands was the Agricultural Revolution, which took place in Great Britain between the seventeenth and nineteenth centuries (Overton 1996). This period saw a shift towards mechanisation and selective breeding for higher yield crops, in addition to a reduction in the amount of agricultural land left fallow, due in part to the introduction of crop rotation (Overton 1996). The first half of the twentieth century saw another leap forward, with the industrialisation of the production of ammonia from atmospheric nitrogen, known as the Haber-Bosch process (Mikkelsen & Bruulsema 2005). This was used to generate artificial fertilisers, allowing for the selection of increasingly fast-growing and high yielding crops (Smil 2002). With the support of initiatives such as the Common Agricultural Policy (CAP) in Europe (European Commission 2008) and 'Farm Bills' in the U.S.A. (National Agricultural Law Center 2003), intensification of farming since the end of the Second World War has thus been characterised by a rise in the use of pesticides and artificial fertilisers, along with a steady increase in the degree of mechanisation (Raun & Johnson 1999, Stoate *et al.* 2001).

Increased output has come a high environmental cost, however. Using the UK as an example, between 1945 and the end of the last century, the number of farms declined by 65% and farm labour by 77%, as productivity rose four-fold (Robinson & Sutherland 2002). Associated with these improvements in yield was the loss of many of aspects of landscape heterogeneity (Benton *et al.* 2003), including around 50% of hedgerows (Robinson & Sutherland 2002), with concomitant declines in soil quality (Loveland *et al.* 2000) and biodiversity in invertebrates (Donald 1998),

birds (Fuller *et al.* 1995) and arable flora (Wilson 1999). There was also an increase in pollution from pesticides, with further associated adverse effects on biodiversity (Wratten *et al.* 1990). Moreover, the last half century has seen the rise of arable agriculture at the expense of pastoral, in part driven by incentives (such as those generated by the CAP) to abandon practices which integrate crops with livestock and forestry, leading to a 92% decline in 'unimproved' grassland between the 1930s and 1980s, with severe resulting impacts on the provision of natural enemies suited to arable crops (Fuller 1987, Gravesen & Toft 1987). Presently, the UK is a net exporter of grain, but recent estimates, however, indicate that when food is considered overall, the UK is a net importer with a shortfall of around 42% of its total food requirements (DEFRA 2010).

1.2. Political and economic framework

Fears that food production will fail to keep pace with rising populations have existed since Malthus (1798) and continue to be a concern (e.g. Meadows *et al.* 1972, 2004, Moore 2010). Not only is food supply threatened by climate change (IPCC 2007, Godfray *et al.* 2010), but the recent spike in fuel costs had knock-on effects on food prices, highlighting a number of factors that indicate that the economic framework in which agriculture operates does little to encourage food security. Commodity markets are poorly regulated (FAO 2009) and are furthermore closely coupled to the cost of fossil fuel energy (Pimentel 2009), the cost of which is likely to become increasingly volatile due to market speculation and the progressively increasing difficulty in its location and extraction (Hirsch 2008). This coupling is likely to have ramifications in the markets on which the inputs to modern farming rely: those of machinery, fertilisers and pesticides. All these factors (and their influences) will alter the cost/benefit equations governing individual farmers' decisions to employ modern agricultural techniques.

Given that the costs of farming are usually framed explicitly in economic terms, it makes sense to define the value of ecosystem services provided by nature in the same terms (Costanza *et al.* 1997, Losey & Vaughan 2006). Ecosystem services are defined as those natural processes which provide basic needs (Millennium Ecosystem Assessment 2005). Many such ecosystem services are provided by non-crop habitats, including fresh water, flood protection, global climate regulation (IPCC 2007), and refugia for pollinators (Allsopp *et al.* 2008) and natural enemies

(Bianchi *et al.* 2006). It is thus imperative to increase the sustainability of agricultural production by reducing exogenous inputs (including the expansion into previously uncultivated land) as much as possible, whilst maintaining output. This is increasingly recognised at a governmental level, both nationally and internationally. Changes to subsidy schemes, for example the introduction of Glastir in Wales (WAG 2010), and legislative action, such as the review of the Council Directive 91/414EEC governing the use of pesticides in the European Union, acknowledge this need.

1.3. Pest control strategies

Since the Second World War, conventional pest control has relied principally on synthetic pesticides, the use of which is difficult to monitor, especially given how weather-dependent application levels are (Stoate *et al.* 2001). However, sales of these chemicals have declined considerably in Europe, particularly since the early 1990s, due mainly to legal restrictions in Finland, Denmark, Sweden and Netherlands (Stoate *et al.* 2001).

Genetic modification may aim to confer a number of different properties to a plant, one of which is the incorporation of pest resistance with a view to reducing pesticide use (Phipps & Park 2002). In the developing world, the decline in pesticide use has indeed been associated with the advent of the genetic modification (GM) of crop plants (Phipps & Park 2002). The efficacy and risks of GM technology have been fiercely debated and remain controversial (e.g. Batista & Oliveira 2009, Abbas *et al.* 2010). *Bt* cotton, for example, uses a gene spliced from the bacterium *Bacillus thuringiensis* to confer resistance to the cotton bollworm, *Helicoverpa armigera*. This modified cotton's deployment on a large scale (along with buffer crops to dilute the effects of the evolution of pest resistance), was found to significantly reduce the incidence of the bollworm pest (Wu *et al.* 2008). However, the emergence of other, hitherto less abundant pests, Mirid bugs (Hemiptera: Miridae), reveals that such species have seemingly benefited from the lack of competition and reduced use of external pesticides conferred by *Bt* cotton (Lu *et al.* 2010). Mirid bugs now threaten to spread further afield than the GM plantations on which they have proliferated (Lu *et al.* 2010).

Another form of pest control is the introduction of non-native (or inundation with native) species that consume pests (so-called biological control). This method has undoubtedly had successes, particularly in the use of predatory coccinellids (Obrycki & King 1998). However, this approach is often risky and has consequences that are difficult to predict and assess (Simberloff & Stiling 1996). A famous example of unforeseen adverse consequences following biological control measures is the introduction of the cane toad (*Bufo marinus*) to North-eastern Australia in the 1930s (Easteal 1981). This species was originally brought to the country to help control sugar cane pests, but it soon proliferated, rapidly expanding its range to the West and South. Moreover, the cane toad proved to be both poisonous to native predatory fauna, and a predator of native species itself, and has long since been considered a pest species in its own right (Easteal 1981). A more ambiguous situation has arisen with the coccinellid beetle, *Harmonia axyridis*. Originating from East Asia, this species was successfully introduced into North America and Europe a number of decades ago, to control aphids and other invertebrate pests, and continues to be broadly effective (Koch 2003). However, *H. axyridis* has since become established outside areas of deployment (Lombaert *et al.* 2010), causing numerous problems due to its consumption of non-pest species, including native coccinellids (Burgio *et al.* 2008, Crowder & Snyder 2010), pathogenic fungus of aphids (Roy *et al.* 2008) plus other aphid predators (Alhmedi *et al.* 2010) and parasitoids (Chacon & Heimpel 2010). Furthermore, *H. axyridis* may have direct detrimental effects on fruit and buildings (Koch & Galvan 2008).

The sustainability of such interventionist methods of pest control is therefore questionable. Integrated pest management (IPM) is a holistic framework aiming to combine traditional methods of husbandry with technologies such as GM and pesticides (Carson 1962, Kogan 1998). The initial and most basic aim of IPM is the exploitation of natural pest control in the most effective way by manipulating the habitat to maximise its potential. Once this initial step has been achieved, the process continues with the quantification of injury thresholds of pests (i.e. the infestation rates below which it is considered uneconomic to employ pesticides) (Johnston & Bishop 1987, Beer & Heitefuss 1981, Larsson 2005). Furthermore, IPM requires that when such chemicals are necessary, they are applied with effective timing (Nicolas 2004, Frihauf *et al.* 2004) and their use on non-crop habitats minimised (Haughton *et al.* 2001).

Conservation biological control (CBC) is the focus of this thesis. CBC aims to identify significant components of natural enemy assemblages in agricultural ecosystems and maximise the economic and environmental benefits that can arise from optimizing natural regulatory systems (Risch *et al.* 1983, Bell *et al.* 2002, Griffiths *et al.* 2008, Tscharntke *et al.* 2008). Assemblages of natural enemies are the foremost defence against pests in arable crops, because these plants have been selectively bred to maximise their nutritional value at the expense of natural pest defences. Organic farming exemplifies the CBC approach and the body which monitors it in the UK, the Soil Association, was established in 1946. The movement has grown considerably in recent decades in reaction to the perceived artificial and harmful nature of conventional pesticides and GM crops (Carsen 1962, Gaskell *et al.* 1999, Batista & Oliveira 2009).

Organic agriculture relies solely on natural methods of pest control, namely CBC, plus non-synthetic pesticides. Bengtsson *et al.* (2005)'s meta-analysis of comparisons of organic versus conventional farming found that in all but 18% of cases, species richness was higher, on average by 30%, on organic farms. Abundances of individual organisms were also higher on organic farms, by an average of 50%, especially among birds, predatory insects, soil arthropods and vascular plants (Bengtsson *et al.* 2005). Comparisons between conventional and organic farms have found superior pest control of pests in both wheat fields (Östman *et al.* 2001a) and orchards (Miňarro *et al.* 2009). Furthermore, evidence exists that the condition of natural enemies in organic systems may be better, in terms of fitness parameters such as bodyweight (Östman *et al.* 2001b).

Organic farms in the UK comprise around 743,000 h (4.0% of the total agricultural land), of which 57,200 h is cereal crops (DEFRA 2010). This is average for the EU and above average for Europe as a whole, the latter of which is 1.9% (FiBL 2010). Among European nations, Spain (1.33m h) and Italy (1.11m h) have the largest areas of orgainc certified agricultural land, while Austria (18.5%) and Sweden (12.5%) and have the highest proportion. Organic farming in the UK is clustered into four main areas, all of which occur in the area South-West of a line stretching from Brighton-Bangor (Ilbery & May 2010). Conversion from conventional farming is ecomonically-driven and more likely to occur in areas where agriculture

is less favoured and so the gains from the addition of economic value to the products and income from agri-environment schemes are more likely to offset the costs of reduced yield (Gabriel *et al.* 2009).

1.4. Historical development of ecological theoretical framework

As new conventions in farming emerged and expanded during the mid-twentieth century, the notion of an idealised abstraction of distinct and functionally homogenous trophic levels acting as part of a linear food chain pervaded (Lindeman 1942). From this perspective, if all trophic levels in a food web are taken into account, the resulting picture usually resembles a pyramid, because energy is attenuated as the food chain is ascended. Lindeman's (1942) theory of "Trophodynamics" stressed the importance of "bottom-up" forces to explain the height and structure of the trophic pyramid, whereby each level of the food web was dependent on the energy available in the level below it, and each level characterised by this flow of energy.

The "green world" hypothesis of Hairston, Smith & Slobodkin (HSS; Hairston *et al.* 1960) challenged this understanding by combining these "bottom-up" (donor-controlled) regulatory mechanisms with the concept of "top-down" (recipient-controlled) effects, whereby herbivore abundance is restricted by predators, allowing producers to thrive, hence our "green world". This theory shifted the focus from the delineation of trophic levels through ascending energy fluxes, by introducing the idea that consumers significantly *regulate* the resource they persist on (Slobodkin *et al.* 1967).

The Exploitation Ecosystem Hypotheses (EEH; Oksanen *et al.* 1981) is a generalisation of the "green world" theory, which specifically describes interactions between three or four discrete trophic levels. Both models assume that each trophic level acts as a single, homogeneous ("exploitative") unit, and both also predict alternating high and low biomass between successive levels. The "bottom-up" element of EEH consists of the prediction that more productive ecosystems (those with higher inputs of energy and nutrients) can sustain a greater number of trophic levels. "Top-down" EEH dynamics predict that where an odd number of trophic levels (i.e. three) exist, producer biomass is high because herbivores are suppressed by predators. These interactions are known as trophic

cascades (TCs), which may be positive (bringing about an increase in producer biomass) or negative (resulting in its reduction) (Borer *et al.* 2005).

Perhaps the clearest examples of TC dynamics can be found in aquatic ecosystems. Estes & Palmisan (1974) demonstrated this phenomenon in pacific kelp forests. The sea otter, *Enhydra lutris*, a specialist consumer of kelp-eating sea urchins, *Strongylocentrotus* spp., is a primary predator in this ecosystem. In places where sea otters have been hunted to extinction, sea urchins have increased in abundance and as such devastate kelp beds in a negative trophic cascade. The EEH predicts, however, that where a fourth trophic level exists (that of secondary predators), this will limit primary predation, resulting in a reduction in herbivore regulation and ultimately, a low producer biomass (Oksanen *et al.* 1981). Carpenter *et al.* (1985) documented an example of this in a study of North American freshwater lakes: piscivorous fish (secondary predators) can efficiently reduce populations of zooplanktivorous fish (primary predators), which in turn can effectively control communities of herbivorous zooplankton. Grazing zooplankton can in their turn have a significant impact on phytoplankton communities' abundance. Experimental removal of piscivorous fish was found to change lake water from clear to green by allowing phytoplankton (producer) to flourish (Carpenter *et al.* 1985).

While strong TCs are well-documented in aquatic systems, evidence from a meta-analysis (Shurin *et al.* 2002) of predator-removal experiments suggests terrestrial TCs are usually weaker than those in aquatic ecosystems. Shurin *et al.* (2002) found considerable variation in the magnitude of predator effects among six types of ecosystem, with the strongest effects in lentic and marine benthos and the weakest in marine plankton and terrestrial food webs. Furthermore, Schmitz *et al.* (2000) found that while TCs did occur in terrestrial ecosystems, their effects were attenuated when herbivore species diversity was high and/or plants had elevated levels of herbivore defence. They demonstrated that vertebrate predators exert stronger cascading effects than their invertebrate counterparts when the system's herbivores are invertebrates, although this was primarily the case where the response was measured in terms of plant damage rather than either plant biomass or reproductive output (Schmitz *et al.* 2000).

Monocultural crops, however, are similar to aquatic food webs, in that they tend to have a low degree of plant diversity. This was shown in a study by Halaj & Wise (2001) who undertook a meta-review of manipulative studies of arthropod-dominated terrestrial systems. They found the magnitude of TCs among crop systems to be greater than that in non-crop systems in terms of herbivore density, plant damage and plant biomass. Halaj & Wise (2001) suggest that the stronger trophic cascades observed in agricultural manipulations than in natural herbivore-dominated systems, are a result of the simple habitat structure and more homogeneous assemblages of plants in arable fields, supporting the original predictions of Root's (1973) "enemies hypothesis". This states that the more diverse resources contained within polycultures may provide greater resources to predators and parasitoids, enabling a more effective functional response by natural enemies. This in turn is hypothesised to make the dominance of particular herbivore species less likely (Root 1973), thus reducing the potential of strong TCs.

1.5. Omnivory and intraguild predation

A parallel development to the theory of TCs and food chains with discrete trophic levels was that of food webs. Early studies (e.g. Pimm & Lawton 1978) used standard Lotka-Volterra models (Lotka 1920, Volterra 1926) to try to assess the effect of omnivory on the stability of four-species trophic modules, concluding that greater levels of connectance lead to less stability in the food web. This was surmised to be because effects could ramify through the system and destabilise it from any point. From this, they concluded that omnivory[1] (i.e. feeding from more than one trophic level) is rare in nature (believing that most reports of it were an artefact of lumping taxonomically similar species together) and characterised by insignificant, weak links (Pimm & Lawton 1978).

Polis *et al.* (1989), however, recognised intraguild predation (IGP), a form of omnivory where species which compete for the same resource also engage in trophic interactions (predation or parasitism) with one another. IGP differs from classical predation in the sense that the act of IGP removes a potential competitor,

[1] 'True' omnivory is defined as consumption from two different trophic levels, one of which is a primary producer (Coll & Guershon 2002). 'Trophic' omnivory consititutes the consumption of a resource from more than one trophic level, regardless of whether a primary producer is involved (Coll & Guershon 2002).

and is distinguished from competition because the IG predator makes immediate nutritional gains (Polis & Holt 1992). IGP may have a number of outcomes; fatality of the intraguild (IG) prey, a behavioural change to the prey through fear (Rosenheim *et al.* 1995) or the expression of a different prey phenotype (Banjeri & Morin 2009)[2]. IGP may be particularly destabilising to a food web, because while nutritional gains from consuming many immature IG prey items may be small, the numerical effect on the population may be large (Polis *et al.* 1989).

The conventions for assessing the effects of IGP on populations were set out by Holt & Polis (1997) who set out a framework for the analysis of trophic modules including omnivory where the relationship between each population is defined by linear Lotka-Volterra equations. They proposed that these equations may be parameterised either at random or using data from field studies. The main aim of such approaches is the assment of the stability of trophic modules in terms of coexistence probabilities under varying conditions. Their main conclusions were that for coexistence of predators to persist i) IG prey must be a superior competitor for the shared prey than the IG predator, ii) an IG predator must gain a fitness benefit from consumption of the IG prey, and iii) the coexistence of IG prey with an IG predator is most likely at an intermediate abundance of their shared resource. At high levels of the shared resource, the IG predator is predicted to dominate (manages to consistently capture the majority of the available resources) or exclude the IG prey to the point of (local) extinction (Holt & Polis 1997, Muller & Brodeur 2002, Kuijper *et al.* 2003). These predictions have empirical support from a number of studies. Morin (1999) and Diehl & Feiβel (2001), for example, have shown these propositions to be true in controlled experiments with ciliates, while Borer *et al.* (2003) identified a natural system in which species populations conformed to these predictions. Of two parasitoids (IG prey and IG predator) of the citrus pest, California red scale, *Aphytis melinus* (the IG predator) dominated at a high resource abundance, while *Encarsia perniciosi* (the IG prey) did so at a low abundance (Borer *et al.* 2003). The two species were most likely to coexist at intermediate density of California red scale (Borer *et al.* 2003).

[2] Lucas (2005) suggests that the definition of IGP be extended to all antagonistic predator-predator interactions (killing and consuming prey, simply killing and non-lethal interference). However, this is potentially confusing, and so I will continue to refer to IGP as the killing and consumption of some part of an IG prey item and non-lethal interference separately.

1.6. Intraguild predation in more complex systems

Crop pest populations often exhibit extreme spatial and temporal patchiness. When such pests represent a trophic resource for a predator, this will lead to widely variable local (i.e sub field-scale) conditions under which IG protagonists coexist despite the seemingly narrow range of conditions where this is predicted to occur (Section 2.2). In the majority of cases, these IG protagonists also provide effective pest control (Bianchi *et al.* 2006, Janssen *et al.* 2006). This gap between theory (section 1.5) and practice has thus recently undergone much scrutiny (Janssen *et al.* 2006). It is increasingly recognised that the three-species models of IGP are oversimplified, as most naturally occurring IG interactions are embedded in larger food webs (e.g. Bascompte & Melián 2005). Further adaptations of Lotka-Volterra methods have therefore been employed to model such scenarios. A pair of recent studies (Daugherty *et al.* 2007, Holt & Huxel 2007) presented more detailed linear dynamic models. Both studies independently made the same predictions that coexistence between IG predator and IG prey is more likely where IG prey can exploit alternative resources (e.g. through cannibalism, or consuming alternative prey, detritus, or pollen) or refugia (either temporal or spatial), but only if the IG predator fails to exploit similar resources (Daugherty *et al.* 2007, Holt & Huxel 2007). These predictions have also stood up to experimental scrutiny. In a microcosm experiment, Shakya *et al.* (2009) examined the effects of combinations of bugs (IG predators) which consumed both thrips (the shared resource) and mites (IG prey), on the abundance of the thrips on strawberry plants. Each of the predators also gained an alternative resource in the form of the strawberry pollen. While the two predators synergistically reduced thrip populations on the flowers of plants lacking pollen, where pollen was present the levels of thrip reduction were no greater than when either predator was present alone. Such antagonism, however, was not apparent on the plants' leaves and fruit, which did not offer pollen as a resource (Shakya *et al.* 2009).

Additionally, theory predicts that weak trophic links (McCann *et al.* 1998) and dietary subsidies such as cannibalism (Rudolf 2007) may encourage coexistence and therefore food web stability. Empirical studies have shown the importance of habitat structure (reviewed in Janssen *et al.* 2007), temporal refuges (Amarasekare 2007) and behavioural mechanisms such as predator avoidance, for coexistence (Folz *et al.* 2006, Lefcort *et al.* 2006, Magalhães *et al.* 2004). Given the ubiquity of

omnivory, including IGP, such interactions are obviously important for the dynamics of ecosystems. Elucidation of their effects would benefit from an approach that considers whole food webs (Woodward & Hildrew 2002, Ritchie & Johnson 2009). The distribution of link strengths (i.e. the levels of consumption by one species of another) and the presence of IGP has also proved an important stabilizing feature in theoretical studies (Gross *et al.* 2009).

1.7. Intraguild predation in studies of pest control

Most prey species suffer predation from multiple predator species (Sih *et al.* 1998). So-called "emergent effects" of predator assemblages (also known as multiple-predator effects, or MPEs) occur when the number of prey consumed in the presence of multiple predator species differs from the summed effects of each predator in isolation (Sih *et al.* 1998, Schmitz 2007). The nature of non-linear effects can be examined with controlled predator-prey experiments (e.g. Sih *et al.* 1998). The basic designs for such experiments are additive (Losey & Denno, 1998, 1999, Finke & Denno 2005) or replacement series (Straub & Snyder 2006)[3], each of which may provide entirely different results (Griffen 2006). Although additive designs confound predator density with diversity, Schmitz (2007) argues that they are useful because predator density and diversity often covary in nature. However, inferences about the type of MPE derived from such experiments should be qualified with a test for substitutability (Sih *et al.* 1998, Schmitz 2007). Predators are assumed to be subsitutable if interspecific IGP or interference is equal in magnitude to intraspecific interactions (Sih *et al.* 1998, Schmitz 2007). A test for substituability requires an estimation of the per capita effect of predators for each experimental treatment (Finke & Denno 2005, Schmitz 2007). Laboratory microcosms are more likely than field experiments to conclude that predator diversity enhances the risk to prey (Schmitz 2007).

A review of studies investigating MPEs (Schmitz 2007) proposes predator and prey habitat domains are an important factor in predicting the effects of predator diversity. The study (Schmitz 2007) identifies generalised scenarios in which

[3] Basic additive design:
[Control - no preds] v [10xPredA] v [10xPredB] v [10xPredA + 10xPredB]

Basic replacement design:
[Control] v [10xPredA] v [10xPredB] v [5xPredA = 5xPredB]

combinations of predators are more or less likely to reduce risk to prey. The relative habitat domains (i.e. the species' usual vertical range in the crop or other host plant) are seen as key to these predictions. In particular, where prey have a broad habitat domain and the domains of predators overlap, the risk to prey are likely to be diminshed as a result of IGP (Schmitz 2007). Given that IGP is considered critical in predicting outcomes of multi-predator interactions, it may confound conclusions derived from experimental treatments by altering the predator or prey densities. It remains unknown whether a predators' numerical response depends more on simple prey density or predator/prey ratio (Abrams & Ginzburg 2000). IGP occuring during controlled experiements is likely to change these parameters, confounding the results of these experiments. Furthermore, sub-lethal predator-predator interactions may change predation rates on prey (Sih *et al.* 1998).

When the effects on the host plant species are also considered, the majority of studies of terrestrial arthropods have shown that increasing natural enemy diversity leads to reductions in pest numbers with a concomitant rise in crop biomass, i.e. a positive TC (Losey & Denno 1998, 1999, Cardinale *et al.* 2003, Snyder *et al.* 2006). Straub *et al.* (2008) provide a review of such studies. Others have produced equivocal results, such as increased pest populations when the ratio of IG predators to herbivore specialists was experimentally increased, but without any increase in plant damage or decrease in biomass (Finke & Denno 2005). Snyder & Wise (2001) found different effects at different stages of the season, while Wilby *et al.* (2005) showed synergistic suppression of just one out of two pest species by the same predator assemblage. Others still have shown a negative relationship between predator diversity and pest suppression (Rosenheim *et al.* 1993, Finke & Denno 2004). Each of these experiments considered IGP to be an important factor, although none directly measured its prevalence (Rosenheim *et al.* 1993, Losey & Denno 1998, 1999; Snyder & Wise 2001, Cardinale *et al.* 2003, Finke & Denno 2004, 2005; Wilby *et al.* 2005, Snyder *et al.* 2006).

1.8. Intraguild predation involving parasitoids

Rosenheim *et al.* (1995) considered predator-predator consumption to be the most disruptive IG interactions between arthropods. This is because developing parasitoids are generally immobile and are thus less likely than mobile prey to be

encountered by a predator. Given, however, that predation on a parasitized pest host does not result in any increase in mortality of the pest population and furthermore removes a potential consumer, this type of IGP may also cause serious disruptions to pest control (Traugott *et al.* 2008). Snyder & Ives (2001) found that predation in an alfalfa crop by *Pterostichus melanarius* initially reduced numbers of the pea aphid, *Acyrthosiphon pisum*, but as the experiment progressed, this process disrupted aphid control by the specialist parasitoid wasp *Aphidius ervi*. This was especially true where plants were short and the beetle could access the aphid mummies (Snyder & Ives 2001). Costamanga *et al.* (2007), however, found no disruptive effects by coccinellids (which are foliar foragers) on aphid parasitoids. Colfer & Rosenheim (2001) examined a system consisting of aphids, parasitoids and coccinellids, finding that the coccinellids did not interfere with parasitoids, but rather showed a partial preference for unparasitised aphids. The combination of these factors was sufficient to ensure that the predators had no negative effect on pest suppression despite high levels of IGP (Colfer & Rosenheim 2001).

1.9. Effects of alternative prey on biological control

The role of 'alternative' i.e. non-pest prey in conservation biologcal control is not simply that of a critical source of alternative prey to sustain early season predators, but also as a potential distraction to pest predation (Bell *et al.* 2008, Kuusk & Ekbom 2010). Such alternative prey are usually detritivores and fungivores. The use of detrital subsidies, then, has the potential to amplify these interactions in either direction (Halaj & Wise 2002, Wise *et al.* 2006). Small spiders, particularly linyphiids and juvenile lycosids, often feed on detritivores (Agustí *et al.* 2003a), and hence may also provide a link between detrital and predatory food web modules via IGP and cannibalism of smaller conspecifics (Halaj & Wise 2002). This is a view supported by Miyashita *et al.* (2003) who showed that preventing the migration of soil arthropods to the forest floor reduced the abundance of generalist predatory spiders, but that this did not subsequently interfere with herbivore suppression. This was perhaps due to a prey switch from detritivores to herbivores by the remaining generalist spiders (Miyashita *et al.* 2003).

1.10. Applying ecological theory to the study system

Annual cereal crops are based on a primary producing species that is bred to be maximally nutritious (i.e. protein-rich) to humans, and as a consequence, to other species in a food web as well. Thus, from the perspective of Exploitation Ecosystem Hypothesis (Oksanen et al. 1981) (section 1.4) they are likely to be sufficiently nutritious to support four trophic levels (Oksanen *et al.* 1981). Indeed, if the crop does not have this potential, it may be considered sub-standard (Basky & Fonagy 2003). Some level of herbivory is therefore inevitable in a high quality crop. In addition to the plant-based channel of the food web, there is also a detritivore-based channel. Aphids represent an important crop pest in cereal crops (section 4.1.3), the commonest of which, at least in North-Western Europe, are the species *Rhapalosiphum padi*, *Sitobian avenae* and *Metapolophium dirhodum*). Natural enemies of aphid pests (see section 4.1.2) range from the specialised, such as hymenopteran parasitoids (Traugott *et al.* 2008), to generalist insectivores, for instance linyphiid spiders (see section 4.1.2), to widely polyphagous carabids beetles, such as *Pterostichus melanarius* and *P. madidus* (see Sunderland *et al.* 1975, Harper *et al.* 2005). More specialised natural enemies are superior to generalists as consumers of aphids (Schmidt *et al.* 2003). However, the actions of generalists early in the season, sustained on non-pest prey from the detrital food channel, play a significant role in delaying and reducing the peak aphid infestations (see section 5.1.1). Semi-field trials of *P. melanarius* (see Prasad & Snyder 2004, 2006, section 3.1.1) and stable isotope analysis of the accumulation of ^{15}N in generalists (McNabb *et al.* 2001) suggest that cannibalism and possibly IGP are present in such food webs.

1.11. The model system, aims and objectives

Wheat is one of the world's most important grain crops, with global per capita consumption of wheat for the year 2003 was estimated at 67 kg (FAOSTAT, UN 2004), with an increasing amount of this crop used to sustain livestock reared for meat production. In this thesis the interactions of invertebrates in food webs on winter wheat (*Triticum aestivum*), which is the most common wheat crop found in North-West Europe, were studied. Particular focus was given to unidirectional IGP between carabids and linyphiid spiders, and carabid predation on their shared aphid prey.

The trophic links amongst invertebrates inhabiting winter wheat fields were studied using post-mortem gut analysis. Polymerase chain reaction (PCR) was used as it provides a means of analysing, at a high trophic resolution, trophic interactions amongst invertebrate communities that are difficult or impossible to study by direct observation (Symondson 2002, King *et al.* 2008). In certain circumstances, comprehensive food webs may be constructed from morphological evidence, such as that of Broadstone stream, an acid headwater of the River Medway in the South-East UK (Woodward & Hildrew 2002). The calcium-rich substrate of this ecosystem ensures that most species have hard parts which survive digestion, allowing trophic links to be identified. However, the absence of such remains, which is the case in most instances of invertebrate trophic interactions, makes PCR a more suitable method. Symondson (2002) reviewed the techniques and studies involving different forms of gut content analysis including PCR, enzyme electrophoresis, mono- and polyclonal antibodies while Sheppard & Harwood (2005) reviewed those studies that employed only PCR. Gariepy *et al.* (2007) gave an overview of the use of molecular markers to study parasitoids in biological control. The study described in the following chapters represents the most intensive attempt to date to establish the direct trophic links between putative generalist IG predators using species-specific PCR.

In the system under study, carabid beetles represent putative intraguild predators (Polis *et al.* 1989, section 1.5) and a number of linyphiid spiders represent putative intraguild prey. Both these groups of species share the wheat aphid, *Sitbion avenae*, as a prey resource. Chapter 2 of this thesis describes the methods employed. These include the dissection of samples, DNA extraction, sequencing, primer design and testing, in addition to experiments assessing the probability of contamination occuring during sample collection. Additionally, two discrete meta-reviews of laboratory feeding trials are reported, with the aim of examining the influence of variables which alter the efficiency of PCR assays, with these variables grouped into those extrinsic and intrinsic to the PCR reactions. Where instrinsic variables are isolated, the hypothesis that fragment size can be used to explain the rate of digestion of a prey DNA fragment was tested.

Chapter 3 describes attempts to identify trophic links among a number of predators (i.e. intraguild predation) that are common in winter wheat in North-Western Europe using PCR to screen the gut contents of predators. In this instance, data from laboratory trials was used to correct for differences in the efficacy of the assays by parameterising Monte Carlo randomisation models to test the null hypothesis that intraguild predation occurs in relation to the abundance of intraguild prey.

Chapter 4 presents an examination, using SADIE (Spatial Analysis by Distance IndicEs) (section 4.3.3) of the interactions between soil properties, aphid abundance and spider populations in a field of winter wheat. The data collection spanned a period encompassing most of the growing season in a field split between two different tillage regimes. This provided the context to implicitly test whether soil properties were affected by the tillage regime and whether this ramified through the ecosystem.

Chapter 5 continues the investigation of IGP and combines predation data with sub-field scale spatial data and analysed using SADIE. Monte Carlo models are again employed to test whether predation is density-dependent, with the investigation widened to two carabid predators' consumption of pest aphids and three species of linyphiid spiders (IG prey). Predation data is also combined with SADIE spatial analysis to investigate the relationship between spatial co-occurrence and predation.

2. Methods and preliminary experiments

2.1. Introduction

Post-mortem gut analysis using PCR (Symondson 2002, King *et al.* 2008a) provides a practical means of analysing, with minimal disturbance to the system under study, trophic interactions among communities that are difficult or impossible to study by direct observation. However, the technique is subject to a number of biases. Sample collection (see Figure 2.1) and preparation (Harwood 2008, King *et al.* 2010a, Weber & Lundgren 2009), DNA extraction methods, the conditions of the PCR assay and DNA visualisation techniques, predator characters such as feeding mode (Greenstone *et al.* 2007) and digestion rates (section 2.7, Lundgren & Weber 2010) as well as primer qualities such as their size and GC (which have a triple hydrogen bond as opposed to the double of AT pairings) content, which influences the temperature at which the primer is predicted to dissociate from its complementary strand (melting temperature, T_m). Additionally, the location of the primers on on the target genome will define the amplicon size (Zaidi *et al.* 1999, Hoogendoorn & Heimpel 2001, Davey *et al.* 2007). All of these factors may influence to some degree the likelihood of whether or not a field-caught predator that has consumed a certain prey item will test positive in a PCR assay. Moreover, precautions must be taken to ensure, as far as possible, the absence of false positives, which may arise through the amplification of non-specific fragments, or from contamination of samples, reagents during collection (Harwood 2008).

This chapter details sample preparation along with the development and testing of the PCR primers used to assay the gut contents of predators in this thesis (sections 2.2 - 2.5). Measures taken to calibrate the use of PCR assays are presented, with a description of controlled laboratory feeding trials employed to establish the persistence of DNA in predators' guts (section 2.6). These results were then analysed in the context of a meta-analysis of all the available feeding trials from the literature, in order to examine the factors influencing variation in this persistence, the results of which are presented and discussed in section 2.7. The methodology and results of field trials to establish whether the collection methods cause contamination are also presented and discussed (section 2.8).

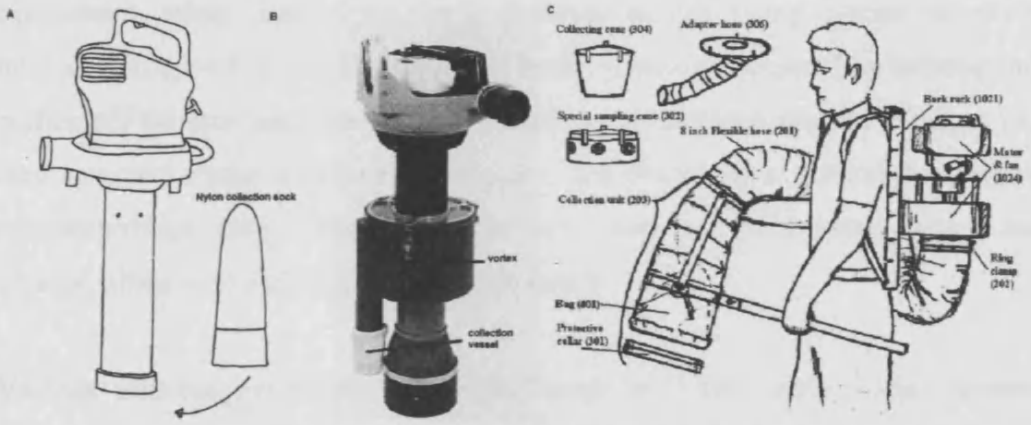


Figure 2.1. Sampling methods for arthropods. A. Adapted leafsucker (also known as G-vac). A garden leaf sucker/blower is adapted by fitting a nylon collection sock to the flange of the sucker. Samples are emptied into a separate bag. B. Vortis sampler (Burkhard Ltd, Rickmansworth, UK). When placed on the ground, sampled organisms are kept in a vortex. Upon removal from the ground, they fall into a removable collection vessel. C. shows a D-vac a purpose-made collection device which works on similar principals to a leafsucker.

2.2. Preparation and DNA extraction of samples for primer design and optimisation

2.2.1. Sample preparation

For sequencing and cross-reactivity testing, DNA was obtained from the appendages of larger predators, namely beetles and spiders. In the case of spiders, the gut may extend into the proximal part of the leg (Foelix 1996) so legs cut distally of the patella were used, to avoid potential contamination of the DNA with gut contents. Small spiders were processed whole, while for larger species (i.e. lycosids) only legs distal to the patella were used for DNA extraction. However, where specimens were collected for the express puropose of cross-reactivity testing, they were starved for up to seven days to ensure digestion of the entire gut contents. Starvation for longer periods was not tenable because death rates of smaller linyphiids began to increase after this time. After such starvation, the whole organism could be used either for DNA extraction or for feeding to predators in decay rate trials (section 2.6).

In obtaining DNA for gut analysis, extraneous body parts were removed. In the case of smaller beetles (e.g. *Trechus* spp. and *Bembidion* spp.), their legs, trochanters, wings and elytra were removed before being placed in sterile microcentrifuge tubes. All large carabid beetle guts were prepared by twisting and pulling off the head and pronotum, to which the gut remains attached. The gut was then removed using watchmaker tweezers and placed in a pre-weighed, sterile microcentrifuge tube. Gloves and tweezers were sterilized using bleach and ethanol, along with flaming between each beetle.

Vacuum sampling (either by leafsucker, Vortis or D-vac) often renders samples morphologically unidentifiable (section 2.8.1). Thus, PCR for identification to species level was also used. Preparation of juvenile spider samples for this involved, in the case of smaller (first and second instar) spiderlings, the removal of abdomens for extraction of DNA from the remainder, whilst just the appendages were removed and used from sufficiently large individuals in order to minimise the potential for contamination with gut contents.

2.2.2. DNA extraction

DNA for sequencing was extracted using the 'solid tissue' protocol from a Puregene extraction kit (Gentra Systems Inc. Minneapolis, PA, USA) while for gut analysis it was extracted according to the 'animal tissue' protocol of the Qiagen DNeasy tissue kit (Qiagen Ltd, Crawley, UK), substituting the Qiagen spin column with one manufactured by Dutscher Scientific (Cambridge, UK). The Qiagen kit was used for the gut analysis, so that results were consistent with other work the research group had undertaken. In the case of DNA for sequencing, there was no need for any consistency, in terms of potential DNA yield, between extractions, so the cheapest method (Puregene) was used. For the gut content analysis, the Qiagen kits provided a consistent method of extraction. Purchasing the reagents in bulk and substituting cheaper spin columns (Dutscher) reduced the overall cost of the gut content extractions while maintaining consistency throughout.

DNA was extracted from juvenile spiders for the purposes of identification (see section 2.2.1) using a chelex-based method: each sample was placed in a microcentrifuge tube with a locking cap (Sarstedt, Nümbrecht, Germany) to which 50 µL of distilled water was added. The sample was then vortexed for ~20 s and

spun in a centrifuge at 15,600 g for 3 min. Following this, 20 µL of Instagene™ chelex matrix (Bio-Rad Ltd, Hemel Hempstead, UK) was added whilst being stirred on a magnetic mixer, before incubation at 55°C for 30 min. The samples were placed in boiling water for 8 min, then spun for a further 3 min at 15,600 g, after which 20 µL of the supernatant was immediately isolated for use in PCR reactions. In all cases, each batch of extractions included a negative control, where a measure of double-distilled water was subjected to the same extraction procedure as the samples.

2.3. DNA sequencing

2.3.1. COI sequences

The universal primers LCO1490 (5' – GGT CAA CAA ATC ATA AAG ATA TTG G – 3') and HCO2198 (5' – TAA ACT TCA GGG TGA CCA AAA AAT CA – 3') (Folmer *et al.* 1994) were used to amplify a ~710 bp fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene for the following predators: *Bathyphantes gracilis*, *Centromerita bicolor*, *Enoplognatha ovata*, *Erigone atra*, 2 x Erigoninae spp., *Meta mengei*, *Neriene clathrata*, *Oedothorax apicatus*, *O. fuscus*, *O. retusus*, *Loricera pilicornis*, *Nabis ferus*, *Notiophilus biguttatus*, *Pachygnatha clercki*, *P. degeeri*, *Pardosa amentata*, *P. palustris*, *P. prativaga*, *Ocypus olens*, *Tenuiphantes tenuis*, 3 x *Tetragnatha* spp. and 2 x *Xysticus* spp. Sequencing PCR reactions were carried out in a total volume of 25 µL consisting of 2.5 µL 10 x PCR buffer (Invitrogen), 0.0625 U *Taq* polymerase (Invitrogen), 2.0 µM $MgCl_2$ (Invitrogen), 0.1 mM dNTP mix (Invitrogen), 1.0 µg/mL bovine serum albumin (BSA) (New England Biolabs, Ipswich, MA,USA), 0.5 µM each forward and reverse primer (Eurofins MWG Operon, Ebersberg, Germany), and 2.5 µL template DNA (1:5 dilution of original extract). After an initial denaturing step at 94°C for 3 min, amplification proceeded for 35 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 45 s and a final extension at 72°C for 5 min, whereafter products were held at 4°C.

PCR products (10 µL) were purified using 0.25 U each of Exonuclease I and Shrimp Alkaline Phosphotase (USB) (New England Biolabs, Ipswich, MA, USA) incubated at 37°C for 45 min and denatured at 80°C for 15 min. Products were sequenced directly using the original PCR primers on a 3130xl Genetic Analyzer

using Big Dye (v1.1) (Applied Biosystems Inc, Foster City, CA, USA) sequencing chemistry in both forward and reverse orientations.

Up to 658 bp of the mtDNA COI gene was successfully sequenced from all 22 species of spiders, three beetles and a nabid bug. In all cases, sequencing was only considered successful when both forward and reverse orientations were in accordance with each other. Sequences were aligned and checked for sequence ambiguities, frame shift mutations and stop cordons, the occurrence of which would suggest a lack of functionality i.e. that these sequences were pseudo genes or nuclear mitochondrial copies (Numts) (Bensasson *et al.* 2001). No evidence of this was found. Sequences were deposited in Genbank (http://www.ncbi.nlm.nih.gov/, accession numbers FJ899797-FJ899834).

2.3.2. 12S sequences

The universal primers SR-J14197 (5' - GTA CAY CTA CTA TGT TAC GAC TT - 3') and SR-N14745 (5' - GTG CCA GCA GYY GCG GTT ANA C - 3') (Simon 1994) were used to amplify a fragment of ~548 bp of the 12S gene (small ribosomal subunit, SSU). Amplification was attempted for 30 individuals from 18 species. In most cases, two individuals were used per species, one from Highfield at Rothamsted and the other from Burdons Farm, Wenvoe (lat: 51.439N, long: 3.271W). When sequencing of only one individual of a species was attempted, its origins are given as Rothamsted (R), or Wenvoe (W). The species sequenced comprised *Bathyphantes gracilis*, *Tenuiphantes tenuis*, *Erigone atra*, *E. dentipaplis*, *Neriene clathrata*, *Oedothorax fuscus*, *O. retusus*, *Centromerita bicolor*, *Meionata rurestris* (R), *O. apicatus* (W), *Xysticus* sp. I (W), *Xysticus* sp. II (W), *Pachygnatha degeeri*, *Pachygnatha clercki*, *Tetragnatha montana/pinicola*, *Enoplognatha ovata*, *Pterostichus melanarius* and *T. monticola/obtusa*.

Sequencing PCR reactions were carried out in a total volume of 25 µL consisting of 2.5 µL 10 x PCR buffer (Invitrogen), 0.0625 U *Taq* polymerase (Invitrogen), 2.0 µM $MgCl_2$ (Invitrogen), 0.1 mM dNTP mix (Invitrogen), 1.0 µg/mL BSA (bovine serum albumin, New England Biolabs, Ipswich, MA,USA), 0.5 µM each forward and reverse primer (Eurofins MWG Operon, Ebersberg, Germany), and 2.5 µL template DNA (1:5 dilution of original extract). After an initial denaturing step at 94°C for 3 min, amplification proceeded for 35 cycles at 94°C for 30 s, 56°C for 30

s, 72°C for 45 s and a final extension at 72°C for 5 min, whereafter products were held at 4°C. 12S sequences were then subjected to the same purification, incubation and sequencing procedures as the COI sequences (section 2.2.1). In most cases, 12S sequencing was only successful in one direction from 5' - 3'.

2.4. Primer design

2.4.1. Species-specific COI primers

The sequences produced here (section 2.3.1), in addition to others taken from Genbank, represented target species, their close relatives and other invertebrates common to wheat fields. All sequences (Appendix I) were aligned using BIOEDIT (Ibis Biosciences, Carlsbad, CA, USA). Alignment began with an initial reference alignment of the whole mitochondrial genome of two spider species, *Habronattus oregonensis* (AY571145) and *Nephila clavata (*AY452691) from which the COI portion of the genome was subsequently excised. Sequences were then added individually, or in groups of two or three closely-related species. The ClustalW algorithm (Thompson *et al.* 1994) set at default gap penalty settings was initially used, followed by manual correction where required. Excess sequence information was trimmed from the alignment (i.e. bases before and after those of the target species' sequence) and primers were designed (Appendix II) and named in relation to their position on each individual species alignment file.

Unique primers of between 18-30 bp were identified on the basis of heterogeneity relative to other sequences (and homology with sequences of all target species for group-specific primers). In particular, differences at the 3' end of the primer were targeted, as this provides a unique initial binding site and helps to ensure specificity (King *et al.* 2008). Properties of the candidate primers were modelled using Netprimer (Premier Biosoft, Palo Alto, USA). This program estimates melting temperature (T_m) using the 'nearest-neighbour' thermodynamic theory (Freier *et al.* 1986), along with probabilities of hairpin formation, primer-primer annealing and self-annealing. For each primer set, theoretically non- or weakly-interacting oligonucleotides were then paired to produce fragments of ~100-300 bp with minimal difference between predicted melting temperatures. Additionally, Netprimer allocates each primer a score (up to 100) based on the criteria detailed above plus a measure of its randomness.

2.4.2. General primers

The 12S mitochondrial gene codes for ribosomal RNA (rRNA), which folds into a secondary structure of 'stem' and 'loop' regions. These stems are highly conserved between species, while the loops are variable in size and nucleotide composition due to insertions and deletions (indels). Efforts at developing secondary structure models have concentrated on the region which codes for domain III of the 12S gene (Hickson *et al.* 1996, Page *et al.* 2002, Carapelli *et al.* 2004, Webb & Moore 2005). These models are based on a combination of comparative sequence analysis and free-energy folding algorithms, such as MFOLD (Hickson *et al.* 1996, Page *et al.* 2000, de los Monteros *et al.* 2003, Carapelli 2004). Functional constraints mean that intraspecific divergence is very low for this gene as a whole (Hickson *et al.* 1996). This makes 12S particularly suitable for the design of group-specific primers (Dodd 2005) which bind primarily to the conserved (stem) regions. Primer design therefore requires robust sequence alignment to ensure correct identification of these homologies for selection of primer sites. Conserved primer sites spanning a number of indels may thus produce fragments which are polymorphic in size among different species belonging to within a particular taxonomic group (Dodd 2005).

An approach similar to that of COI primer design (section 2.3.1) was used to design a general linyphiid primer targeting domain III of the SSU (12S). Sequences representing common arable invertebrates were downloaded from Genbank (Appendix III) along with those species successfully sequenced (section 2.3). Initially, the entire sequences were aligned, after which domain III was isolated (positions 1,174-1,477 in the human sequence of Anderson *et al.* [1981]). Next, a manual alignment was made of non-target species (Appndix '12s alignment'), followed by the new linyphiid spider sequences (section 2.2.2). This process was aided by the general invertebrate secondary structure model of Hickson *et al.* (1996), used to infer the position of stems and loops. This alignment was manually converted to a FASTA file and loaded into BIOEDIT. Once a few spider species were aligned with the non-target species, it was possible to automatically add further spider species with little manual correction required.

A single primer site on the 12S gene (domain III) was identified where linyphiid sequences were homologous for all but one base. At this site, however, one allele

was homologous for the Linyphiinae and one for the Erigoninae. It thus provided a unique non-degenerate primer site for sub-family level discrimination, and a primer site with one degenerate base for a general linyphiid-specific primer. This primer has remained untested, but is detailed in Appendix III along with the 12S alignment.

2.5. Primer testing and optimisation

2.5.1. Species-specific primers

Selected primers were empirically tested across a range of annealing temperatures and concentrations of reagents. Those which produced strong amplification at high annealing temperatures (≥ 58°C) were selected. Initial non-target testing for cross-amplification (using five or six species most closely related to the target) was carried out in parallel with this selection procedure, ensuring specificity was not compromised. The concentration of target and non-target DNA was estimated by taking duplicate readings of each sample using a NanoDrop spectrophotometer (ThermoScientific, Wilmington, DE, USA). This was then diluted to the same concentration as target template DNA (~10 µg/mL), which was the concentration used consistently throughout the process of optimisation. A standard initial PCR was undertaken using template DNA of the target species across a gradient of annealing temperatures in 10 µL reactions, each comprising: 2.0 µM $MgCl_2$, 0.2 µM of each dNTPs, 0.5 µM of each forward and reverse primer, 0.1 µg/ml BSA, 0.0625 µL of Taq polymerase (Invitrogen) and 1 µL of DNA template. PCR was run at an initial denaturing temperature of 94°C for 3 min, then for 35 cycles of: 94°C for 30 s, 55-68°C for 30 s, 72°C for 45 s, before a final extension of 72°C for 5 min, after which time products were stored at 10°C in the thermocycler, before being refrigerated at 5°C until electrophoresis. Amplification was then attempted across a range of concentrations of $MgCl_2$, primer and dNTPs, at an annealing temperature deemed optimal. At this stage of optimisation, template DNA of closely-related, non-target species and negative controls were also included.

Once optimal conditions for each primer set were established, non-target testing (Admassu *et al.* 2006, King *et al.* 2008) was carried out against 119 different prey species/groups from 11 orders, including eight families of spiders (Appendix IV), with each reaction containing ~15 µg/mL of predator DNA.

Diagnostic PCRs were carried out in 10 µL reactions, on 96-well plates in a Bio-Rad DNA Engine Peltier thermal cycler (Bio-Rad, Hercules, CA, USA) and subjected to the following cycling parameters: initial denaturing temperature of 94°C for 3 min, then 35 cycles of: 94°C for 45 s, primer-specific annealing temperature for 45 s, 72°C for 1 min, then a final extension of 72°C for 5 min, after which products were stored at 4°C. Primer sequences, species-specific annealing temperatures and PCR mixes are detailed in Table 2.1.

Target species	Primer names	Fragment size (bp)	PCR conditions					
			Ta (°C)	[$MgCl_2$] (µM)[1]	[dNTPs] (µM)[1]	[Primer] (µM)[2]	Taq (U)[1]	BSA (µM)
Bathyphantes gracilis	B_grac-C1-80F B_grac-C1-266R	187	62	3.0	0.02	0.20	0.0625	1
Bathyphantes gracilis	B_grac-C1-163F B_grac-C1-350R	188	60	3.0	0.03	0.25	0.0625	1
Bathyphantes gracilis	B_grac-C1-226F B_grac-C1-350R	125	60	3.0	0.03	0.20	0.0625	1
Tenuiphantes tenuis	T_ten-C1-153F T_ten-C1-450R	298	61	3.5	0.03	0.15	0.0625	1
Pachygnatha degeeri	P_degeeri_C1-277F P_degeeri_C1-596R	318	64	3.0	0.02	0.15	0.0625	1
Erigone atra & E. dentipalpis	Erigone_sp_C1-146F Erigone_sp_C1-389Rb	244	62	3.5	0.02	0.20	0.0625	1
Erigone atra	E_atra-C1-F1 E_atra-C1-R1	247	59	3.5	0.02	0.20	0.0625	1
Erigone dentipalpis	E_denti-C1-F1 E_denti-C1-R1	247	58	3.0	0.02	0.20	0.0625	1
Pardosa spp.	Pardosa_C1-255F Pardosa_C1-593R	285	57	4.0	0.02	0.20	0.0625	1

Table 2.1. Primer sets optimised for singleplex PCR reactions. Cycling parameters are detailed in section 2.5.1.

2.5.2. Multiplex PCRs

Candidate species-specific primers for optimisation in multiplex reactions were selected so that the differences between their predicted melting temperatures were minimal, and so that an even spread of fragment sizes would allow discrimination of the resultant fragments on agarose gel. Multiplex PCR has been shown to be an efficient high-throughput method of detection of large numbers of prey species (Harper *et al.* 2005, King *et al.* 2010a). Screening in multiplex is based on a commercial kit designed originally for the amplification of multiple microsatellite loci. It is important, however, that a multiplex PCR reaction is capable of

amplifying potential prey in all combinations, and that this is done without the creation of phantom bands due to non-target bonding of a forward primer designed for one species combining with the reverse of another. This is particularly crucial when screening generalist predators that may contain multiple prey items in their guts simultaneously. Multiplex PCR reactions may potentially amplify n^2-1 combinations of target prey. Additionally, there exists the possibility of preferential amplification of one fragment over another. Minimising the number of primer pairs in a reaction reduces the magnitude of such bias. Thus, in no case was an attempt made to optimise a multiplex reaction designed to amplify more than four species simultaneously (see below). Standard cycling parameters were used throughout the process of multiplex optimisation; after an initial denaturing step at 94°C for 15 min, amplification proceeded for 35 cycles at 94°C for 90 s, various annealing temperatures for 45 s, 72°C for 45 s and a final extension at 72°C for 10 min, whereafter products were held at 10°C in the cycling machine before transfer to the refrigerator at 4°C.

Four multiplex reactions were successfully optimised to conform to the requirements outlined above (Appendix V). The first reaction successfully amplified fragments of *Erigone* spp. (205 bp), *Tenuiphantes tenuis* (145 bp) and *Bathyphantes gracilis* (271 bp). A second multiplex amplified *Pachygnatha degeeri* (123 bp), *Oedothorax fuscus* (149 bp) and *Oedothorax retusus* (300 bp). These two reactions were subjected to cross-amplification testing on a wide range of species at these optimal conditions (Appendix IV).

Another two multiplex reactions were optimised, but not used. Given the inconsistency of the results of the dropout testing with four primers and four species present (Appendix V), it was decided to limit the number of species in a multiplex to three. In this case, while no species failed to amplify, the necessity of quadrupling the concentration of one primer (see Figure 5 of Appendix V) in order that all four species could be amplified simultaneously, seemingly resulted in preferential amplification of certain fragments.

2.5.3. Results of primer testing

All of the primers tested successfully amplified the target DNA fragments, without amplifying non-target DNA. The taxonomic resolution at which it is possible to detect prey remains using PCR depends on the genetic diversity of the target gene. In some cases, species will be morphologically distinct (as adults if not at earlier stages of life), on the basis of primary sexual characteristics, and are reproductively isolated, but nevertheless still have low COI diversity e.g. *Erigone* spp. (Araneae: Linyphiidae). This lack of diversity precluded the design of unique primers which would work efficiently enough to distinguish the species in PCR reactions, with annealing temperatures sufficiently high to ensure their specificity. Triple-bonded C and G bases allow the design of primers with higher melting temperatures, thus permitting PCR reactions to be run at higher annealing temperatures. Unfortunately, many arthropods possess AT-rich mtDNA, which compounds the problem of limited genetic diversity, as unique sites available are often AT-rich. Other taxa, such as earthworms, have recently been shown to possess a large measure of cryptic diversity, with morphologically indistinguishable 'species' possessing a wide diversity at the target COI gene (King *et al.* 2008).

2.6. Feeding trials – estimating amplicon decay rates during digestion

Controlled feeding experiments were used to test the ability of each primer pair to detect semi-digested prey DNA in predator's guts over time. *Pterostichus melanarius*, *P. madidus* and spiders were collected from Burdon's Farm, Wenvoe, between July 2007 and August 2008. Beetles were captured in small (9 cm Ø) pitfall traps and maintained individually in 12 x 6 cm clear plastic tubs containing ~50 g of moist peat. Spiders were collected by either pitfall trapping or leafsucker, and maintained in 5 cm petri dishes filled ~1 cm deep, with saturated plaster of Paris mixed with charcoal to maintain humidity. In the weeks before the trials began, standardised predator feeding regimes and subsequent starvation times accommodated potential changes in regulation of digestive physiology, such as levels of DNAases, about which little is known in invertebrates (Schernthaner *et al.* 2002). Thus, the beetles were fed one final instar *Calliphora* sp. maggot (obtained from fishing tackle shops) on the day of capture, then subsequently one maggot per week for three to four weeks. Beetles and spiders were maintained on a 16:8 light: dark cycle at 16 ± 1°C. Beetles were starved for 14 days prior to the feeding trials and spiders for seven days. Both were subsequently killed by freezing at -80°C.

For each feeding trial, 106 *P. melanarius* were presented with a single starved spider in a 90 mm petri dish lined with a sheet of damp 85 mm filter paper approximately 1 h after the onset of the dark phase of their day/night cycle. Five beetles were killed after the 14 day starvation period as unfed controls. Prey items were presented for a 2 h period, the midpoint of which was designated as T_0 (the mean point at which consumption occurred). During this 2 h feeding period, prey consumption was monitored every 15-20 min. Once the beetles had fed, filter paper was removed from the petri dish to eliminate the possibility of the beetle's ingesting any remains of the prey further into the experiment. Those beetles which fed were divided into cohorts and allowed to digest their spider prey for 1, 3, 6, 12, 18, 24, 36 and 48 h following T_0 at a temperature of 16 $\pm$ 1°C (for the *Erigone* spp. feeding trial the 3 h digestion period was replaced with a 62 h sample. This maximum digestion period was extended after finding that *T. tenuis* was still detectable after 48h). Only those beetles that fed were used for the experiment. Each beetle was killed by freezing at -80°C after being placed head first in a 1.5 mL micro centrifuge tube (Fisher Scientific, Loughborough, UK). In most cases, ten beetles per time period were analysed for *Erigone* spp., *T. tenuis* and *P. degeeri*, and eight per time period for *B. gracilis* (Appendix VI). As more males were caught in pitfall traps than females, only two to three female beetles were used for each time cohort in the feeding trials.

2.6.1. Statistical analysis of feeding trials

Feeding trials were analysed in a manner specific to the study systems and presented in the relevant chapters (section 3.4.2). Here, an overview and statistical analysis of these, along with feeding trials from the literature, is presented. The conventional measure of decay in detectability of fragments in comparisons of predation by different species or using different primers is the median detection period. This is the point at which the target fragment is likely to be amplified from 50% of the predators tested (hereafter referred to as T_{50})[4]. This was estimated for

[4] Preferences vary as to the nomenclature of the median detection period in relation to prey detection feeding trials. In some cases, half-life is preferred (e.g. Greenstone *et al.* 2010 and references therein), while in others median detection time (MDT) is given (e.g. King *et al.* 2010b). However, the T_{50} is used herein, because it is compatible with changes in the point at which one

each feeding trial by fitting a logistic (binomial) regression model to the results and interpolating the number of hours post-feeding for the 50% value by rearrangement of the regression equation. Analysis was carried out in R version 2.10.1 (R Core Development Team 2010) using the MASS library (Venables & Ripley 2002).

The binomial model ensured that for any value of the explanatory variables, the response variable (the proportion of successful amplification attempts) were in the range $0 < 1$, sample size data were retained (i.e. the number of successes and failures were included in the model, not simply the proportions) and non-normal (binomial in this case) error distributions were accounted for (Crawley 2007). Plots of the rates of decay revealed that while a number were exponential, others had a straight line relationship. So in deriving the T_{50}s, in each case a log-transformation of the explanatory variable (time post-feeding) was compared to the untransformed model, and the best fit to the data was established by the models' AIC value (Akaike Information Criteria). The binomial model was considered an adequate description of the data when there was no overdispersal, i.e. the residual scaled deviance did not exceed the calculated degrees of freedom. Where a model was overdispersed, the binomial link function was replaced with a quasibinomial, which corrects for overdispersion through the introduction of a scale parameter, making the calculation of an AIC value impossible. In these cases, the selection of best fit was performed by visual matching of the model to the data.

2.6.2. Review of feeding trials

Two meta-analyses of feeding trials available from the literature were undertaken to identify the main factors contributing to the variation in prey DNA decay rates in order to predict reasonable correction factors. Such correction factors may be used to weight the trophic interactions established by species-specific PCR primers when no feeding trials have been performed. The meta-analyses were limited to those feeding trials for which the sample size and number of positives for each time period were available, because the logistic regression required the input of a vector

---

interpolates the T-value e.g. T_{90}, and is consistent with the similar LD_{50} (median lethal dose) terminology from which the statistical model is derived.

containing both successes[5] (number of PCR negatives) and the number of amplification attempts in a batch.

The meta-analyses were carried out as follows. First, the T_{50} of each feeding trial for which suitable data were available was estimated, as described above (Appendix VI). These T_{50}s were entered into the model as the response variable, and the data analysed with a linear mixed effects model (LMM) (Pinheiro & Bates 2000) using the R package 'nlme', (Pinheiro *et al.* 2009). The identity of the 'predator' and that of the 'prey' were set as random effects, with 'prey' nested within 'predator' (Appendix VII). The main fixed effects analysed in the model were the feeding mode of the predator ('mode'; either chewing followed by ingestion of the whole prey item, liquid feeding or extra-oral digestion followed by ingestion), the size of the fragment being amplified ('frag'), the annealing temperature of the PCR reaction ('T_a') and the temperature at which the feeding trial was run (i.e. the ambient temperature at which the predator digested its prey).

Stepwise removal of non-significant terms was undertaken to establish the minimum adequate model, i.e. a parsimonious fit to the data with only significant explanatory variables included (Crawley 2007). Initially, a maximal model consisting of all the explanatory variables and interactions was run. Interactions of an order greater than two-way were excluded, as these were not thought to be biologically meaningful, and their inclusion could have led to multiplicity of P values (Grafen & Hails 2002). Non-significant variables were removed from the maximal model, starting with the least significant highest order terms. An ANOVA was performed at each stage to ensure that the removal of these terms did not significantly reduce the overall explanatory power of the model. This procedure was repeated until the minimal adequate model was obtained. The final model was then subjected to visual checks for heteroscedasticity, non-normality of errors and leverage (Crawley 2007).

An attempt was also made to examine which factors intrinsic to the PCR were the most influential in predicting whether a predator will test positive for a target prey

[5] So-called by convention because the statistical terminology originates from theories of gambling (Crawley 2007). So, strictly, a PCR failure is called a success, in the same sense that a death is also a success in an experiment to establish the efficacy of a pesticide using LD_{50} as a measure.

DNA fragment. Thus, the results of a subset of the feeding trials (Appendix VIII) all of which used *Pterostichus* spp. as predators, and were all subject to the same experimental protocol including the ambient temperature at which the beetles were allowed to digest their meals. T_{50}s interpolated from a binomial regression were again used as the response variable. A linear regression was run with the following factors as explanatory variables: the predator-prey mass ratio ('ratio'), fragment size ('bp'), the maximum difference between the PCR annealing temperature and the predicted melting temperature of either of the primers ('Ta_max') and whether the feeding trial was screened with a multiplex reaction which contained other primers.

2.6.3. Results and discussion of meta-analyses

The meta-analysis of all the feeding trials showed that ambient temperature of digestion was the only variable which explained the variation in decay rates. While this factor was only marginally non-significant (LMM, $F_{1,16} = 4.40$, $P = 0.0522$), its removal was not justified, as doing so caused a significant decline in the model's explanatory power (L-ratio - 3.99, $P = 0.0456$).

Analysis of the subset of *P. melanarius* feeding trials showed that a minimal model retaining fragment size was significant (linear regression, $F_{1,13} = 5.711$, $r^2 = 0.3052$, $P = 0.0327$) (Fig. 2.1).

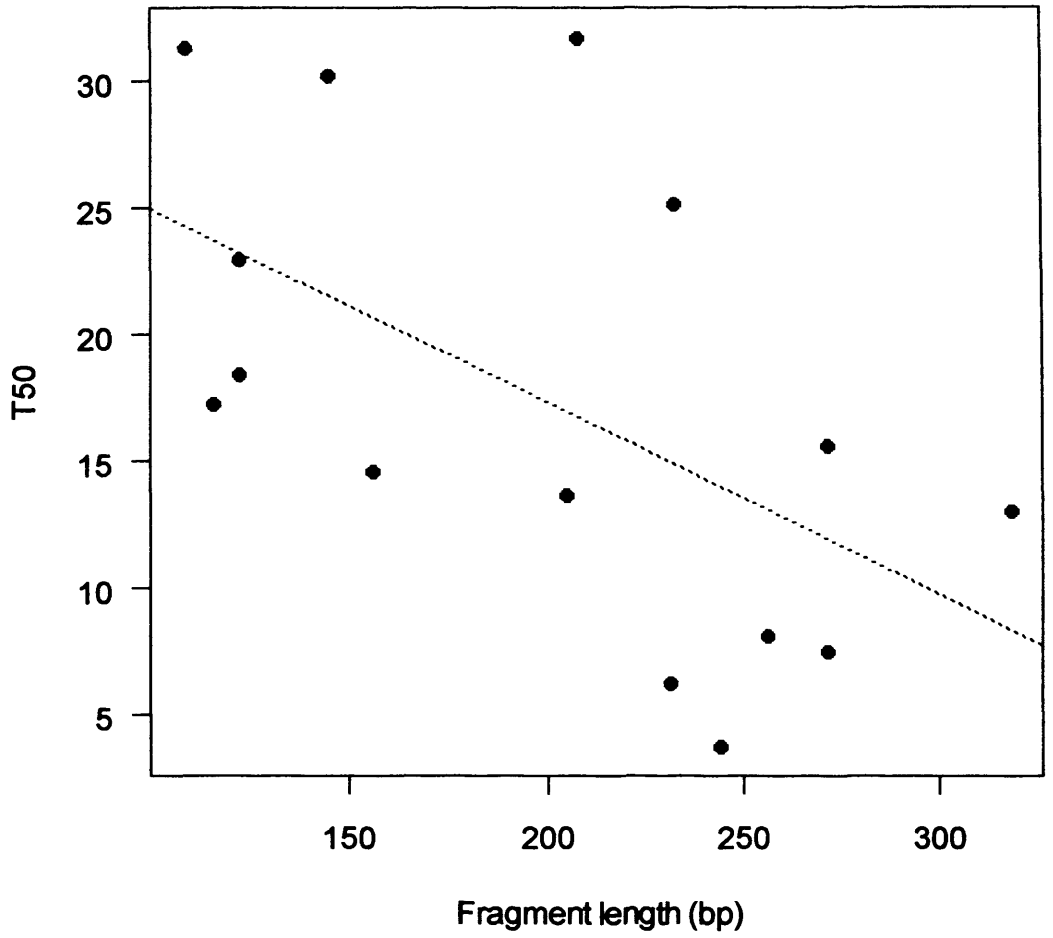


Fig. 2.1. Stepwise regression analysis of a range of T_{50}s derived from feeding trials performed to assess the decay in detection of various fragments of prey in the guts of *Pterostichus melanarius*. Fragment size was the only significant factor (Linear regression, F = 5.711, r^2 = 0.3052, P = 0.0327).

The significance of the temperature at which a predator digests its prey has a number of implications. It can be difficult to establish whether a species exhibits thermotaxis, or simply tolerates a range of temperatures. In invertebrates, it is likely that the latter, i.e. eurythermy, is the case (Lovei & Sunderland 1996). It follows that feeding trials run at temperatures that fluctuate, mimicking those of the habitat under study, i.e. in 24 h cycles (e.g. Chen *et al.* 2000, Greenstone *et al.* 2007), may be more realistic than those undertaken at a constant, arbitrary temperature. However, carrying out feeding trials in this manner (cyclical temperatures) may bias the observed T_{50}s. Species that digest their prey (or more precisely the target DNA fragment) rapidly, e.g. before a daily temperature cycle is

complete, would do so at a different ambient temperature from one that takes a whole cycle to digest. Synchronisation of the predator species' diurnal feeding times in order to reflect their likely natural digestion times would solve this issue, but this relies on the elucidation of these diel activities. The absence of any interaction between ambient temperature and any of the other variables in the full meta-analysis suggests that potential biases caused by the temperature cycles, are not a critical issue. Nevertheless, such interactions might still be possible, so feeding trials run at a constant, arbitrary temperature remove this potential source of error. This pragmatic view accepts the artificiality of laboratory feeding trials, and that these conditions provide a more robust *comparative* measure of differential rates of decay in detectability than might be produced by attempts to render the experimental set-up more realistic.

With a fuller gut, predators may digest food more slowly. Little enzymatic digestion takes place in the foregut of *P. melanarius*. If the food moves out of the foregut at constant rate, then a fuller crop will test positive for longer, not due to a lack of digestion *per se* but simply residence time. The only explicit test of this was carried out by Harper *et al.* (2005), who demonstrated that a fragment of the slug *Deroceras reticulatum* was detectable for a significantly longer period in the guts of *P. melanarius* that had been fed a chaser prey of *Calliphora* spp. than in those that had not (Appendix VI). Starvation may also act to lengthen persistence of prey DNA in the gut, by acting to decrease the predators' metabolic rates to maximise the nutritive value of what it perceives as scarce prey, especially in spiders (Foelix 1996). Variation in satiation is wide. Beetles are often food-limited in the field (Lövei & Sunderland 1996, Bilde & Toft 1998, Powell *et al.* pers. comm.), yet may show evidence of having consumed multiple meals during short periods (King, pers. comm.). Such interactions confirm that feeding trials are simply *relative* measures of prey decay. Laboratory feeding trials are a costly and time-consuming process, so their value in unravelling the effects of this multitude of factors must be weighed against the opportunities for making more trophic observations in the field, albeit with less robust methods of bias correction.

A pragmatic approach to examining the balance between effort and accuracy in applying correction factors was investigated here by comparing a number of feeding trials using the same predator and a range of prey. The results of the *P.*

melanarius meta-analysis show that the only significant variable affecting the decay in detectability of a target amplicon was the size of the DNA fragment (Fig. 2.1). Thus, for trophic interactions between *P. melanarius* and prey for which no feeding trials have been performed, using such estimated correction factors may serve as a pragmatic solution to adjusting for PCR assay bias. This is especially pertinent given the rarity of many prey species, especially in arable ecosystems (Downie *et al.* 1996), and the broad trophic niche of these predators (*P. melanarius* has, to some degree consumed all 22 trophic species for which they were screened; King *et al.* unpublished data.). Thus, with the caveat that the other variables in the PCR assay, such as the fragment size and annealing temperature are within the range of those included in the analysis, T_{50}s interpolated from this model may serve as correction factors with known errors (Appendix VI) for studies of relative predation rates. Research in this area could be directed to establishing the actual temperatures at which target predators are attracted. If such temperatures were of a narrow range, then the small range of temperatures analysed would prove sufficient.

2.7. Sources of error - PCR contamination during sample collection

The use of standard collection methods for population estimates such as sweep-netting, pitfall-trapping (King *et al.* 2010a) and vacuum sampling with both purpose-made D-vacs, Vortis samplers (Burkhard Ltd, Rickmansworth, UK) and leafsuckers all risk introducing contamination during sampling. This may occur due to the surface contamination of a specimen as a result of damage to another specimen, which is more likely in the case of vacuuming with a leaf-sucker. 'Spurious' predation within the collection container during transfer to a suitable storage medium (King *et al.* in prep), meanwhile, is a source of false positives more likely with Vortis sampling than a leafsucker. Placing samples *en masse* directly into alcohol has the potential to preserve more genetic material in the gut for longer (Weber & Lundgren 2009), but risks topical contamination from the trapped animals, which may vomit their gut contents into the medium due to stress (Bell *et al.* pers. comm.). The alcohol subsequently fixes the DNA on the exterior of the predators, preventing the possibility of washing off the contaminating DNA. Harwood (2008), however, found that samples gathered by sweep-netting were no more likely to test positive for prey than those collected by hand using a pooter.

2.7.1. Assessment of sampling contamination - methods

A potential solution was identified to the problem of effectively sampling of small arthropods (see Elliot *et al.* 2006) whilst preserving their suitability for molecular screening. D-vac sampling has proved efficient in terms of providing meaningful data to estimate populations of small arthropods (Elliot *et al.* 2006). With the force of suction still acting on live specimens in the collection bag of a leafsucker or D-vac unit, spurious predation (i.e. consumption that takes place in the collection vessel) was considered less likely than Vortis sampling, which deposits the samples in a collection cup. Dry ice can be easily transported to field sites. The surface temperature of dry ice is approx -75°C and therefore sufficiently cold to kill specimens almost immediately, preventing spurious predation and delay in preservation of the samples[6]. A disadvantage of using a leafsucker or D-vac in comparison to a Vortis sampler is that it subjects the specimens to more damage, raising the potential of topical contamination from the gut contents and haemolymph of damaged specimens.

A field study was undertaken to qualify the assumption that small arthropods collected by leafsucker would not result in either topical contamination or spurious predation. As the focus of this study was intraguild prey, a number of linyphiids and *Pachygnatha degeeri* were collected by vacuum sampler from Burdons Farm, Wenvoe, identified and starved for seven days in 5 cm petri dishes with damp plaster of Paris, at 16°C to ensure their guts were empty. A subset of these spiders were killed as negative controls, while a number (Appendix IX) were labelled with acrylic paint and released back into the field, whereupon attempts were made to immediately recapture them. This was done using an adapted leaf-sucker with a ~200 mm Ø nozzle fitted with a fabric collection 'sock', on full power for 15 s. Sampled specimens (which included diptera larvae, collembola, small carabids, Nabidae, aphids, linyphiid and Tetragnathiidae spiders among other species) were then immediately transferred collectively to small plastic tubs half filled with dry ice (-78°C). Care was taken to ensure the spiders remained under suction to inhibit potential predation in the collection sock and thus ensure that any amplification was due to topical contamination. Once emptied onto the dry ice the samples were gently shaken to ensure all specimens were promptly frozen. Activity was observed

[6] There is a risk that molluscs may explode when introduced to such low temperatures (-78°C) (Symondson, pers. comm.) but vacuum sampling is inefficient at sampling these species.

to cease almost immediately (< 5 s). This process was duplicated, one batch of predators were released and recaptured at the field edge, one at a point 10 m from the crop edge. The method provided approx 20 - 40 minutes for transportation, until temperature in the tubs rose above freezing, allowing transfer to a domestic freezer located ~1 km from the collection site. All samples were transferred to this freezer (-10°C) before being packed into more dry ice for transport back to the laboratory the following day, whereupon they were placed in a freezer at -80 °C.

DNA was extracted from the spiders using the Qiagen DNeasy tissue kit (Qiagen Ltd, Crawley, UK), substituting the Qiagen spin column with one manufactured by Dutscher Scientific (Cambridge, UK), allowing an extraction negative for each batch of extractions. The spiders were subsequently screened for prey items they may potentially have consumed and/or been contaminated with during the experimental procedure. These included spiders with the 'COM LINY MPLEX' and the 'OEDO MPLEX' (Appendix V), at the PCR conditions outlined in Appendix V (which also served as an extraction positive for each spider). Additionally, each spider was screened for diptera of the order Cyclorrhapha and the carabid beetle *Notiophilus bigutattus* using a duplex reaction (King *et al.* 2010a). Amplification of a 177 bp fragment of collembola was attempted using the general 18S primers Col4F (5' - GCTACAGCCTGAACAWTWG - 3') and Col5R (5' - TCTTGGCAAATGCTTTCGCAGTA - 3') (Kuusk & Agustí 2007). For the collembola, PCR reactions were carried out in a total volume of 10 µL consisting of 1.0 µL 10x PCR buffer (Invitrogen), 0.0625U *Taq* polymerase (Invitrogen), 3.5 µM $MgCl_2$ (Invitrogen), 0.02 mM dNTP mix (Invitrogen), 0.1 µg/mL bovine serum albumin (BSA) (New England Biolabs, Ipswich, MA, USA), 0.2 µM of each forward and reverse primer (Eurofins MWG Operon, Ebersberg, Germany), and 1 µL template DNA. After an initial denaturing step at 94°C for 3 min, amplification proceeded for 35 cycles at 94°C for 30 s, 59°C for 30 s, 72°C for 45 s and a final extension at 72°C for 5 min, whereafter products were held at 10°C. Finally, amplification of aphid DNA was attempted using the COII primers AphidF (5'-TTTCCGATTAATTGAAGTAG-3') and Aphid R (5'-ATTCCTGGTCGGTTTATAAA-3'). Optimisation of this primer pair revealed that it amplified only a 181 bp fragment of *Rhapalosiphum padi*. Additionally, therefore, attempts were made to amplify a 110 bp fragment of *Sitobion avenae* using the primers C1-J-2023 (5' -TGATCAATYTTAATTACAGC-3') (Read 2007)

and C1-N-2158 (5'-AATCAAAATAAATGTTGATA-3') (Harper *et al.* 2005). Each of the aphid primers was run in 10 µL reaction containing 5 µL of multiplex master mix (Qiagen), 1.0 µL 'Q' solution (Quiagen), 1.0 µg/mL bovine serum albumin (BSA) (New England Biolabs, Ipswich, MA, USA), 1.0 µM each forward and reverse primer (Eurofins MWG Operon, Ebersberg, Germany), and 1.0 µL template DNA (1:5 dilution of original extract). at the following PCR conditions: After an initial denaturing step at 94°C for 3 min, amplification proceeded for 35 cycles at 94°C for 30 s, 57°C (*R. padi*) and 59°C (*S. avenae*) for 30 s, 72°C for 45 s and a final extension at 72°C for 5 min, whereafter products were held at 10°C.

2.7.2. Results and discussion of sampling contamination experiments

Of the 32 spiders marked and released, 19 were recaptured. Of these 19, none tested positive for any other prey than collembola, for which nine spiders tested positive for a 177 bp fragment (Appendix IX). Each of the starved controls (two individuals of each species) tested negative for each of the amplicons, suggesting that these positives were not the result of prey remaining in the guts of the predators for the duration of the experiment. Furthermore, negative extraction controls ensured that contaminated reagents were not responsible. A small possibility exists that in the few seconds following release, some of the spiders may have consumed prey, but given the precautions that were taken to ensure this did not occur, the most likely explanation remains that the collection method caused topical contamination of the predators. These results point to the need for more sensitive collection methods. This could include replacement of the collection sock between sampling occasions; general primers would cause a higher likelihood of amplification if DNA of past collections resided on the collection sock. Increasing the size of the nozzle and/or lowering the suction power, although reducing the probability of capture of some samples would serve to ensure those samples captured would be less likely to be damaged and thus contaminate each other. Placing samples into alcohol should be avoided where one suspects external contamination with DNA because this may have the effect of fixing the DNA to the sample. Washing the exterior of organisms is also a promising avenue of research in order to address the problem of topical contamination and ensure this source of error is isolated from spurious predation (Remen *et al.* 2010, Greenstone, pers. comm.).

3. Unidirectional intraguild predation of linyphiids by *Pterostichus melanarius* in winter wheat along a vertical spatial niche

Abstract

Arthropod predators provide a valuable ecosystem service by suppressing crop pests. However, intraguild predation (IGP), where predators compete for the same prey resource whilst predating on each other, may destabilise population dynamics and potentially increase the risk of pest outbreaks. Unidirectional IGP amongst generalist aphidophagous predators was quantified in a field of winter wheat in the southern UK. Predation was measured on spiders by the nocturnal epigeal predator *Pterostichus melanarius*. The hypothesis that spiders whose spatial niche overlapped most with that of the beetle would be consumed preferentially was tested. Furthermore it was predicted that spider species within reach of the mainly ground surface-active carabids would be consumed more frequently than those with webs higher in the crop. *P. melanarius* were pitfall-trapped over three months and 622 individuals were screened for the remains of four spider species using species-specific PCR primers. Spiders were collected using vortis sampling and juveniles identified by PCR. Three Monte Carlo simulation models were employed to assess whether spiders were consumed disproportionately. One model (Naïve ALL) simply compared the proportions of predators scoring positive for each spider species with relative prey abundances. The second (Decay ALL) incorporated two additional factors: differential rates of decay, during digestion, of each target DNA amplicon, established from laboratory feeding trials, and circadian activity-density patterns of the beetle. The third model was identical to the second, but omitted the inclusion of non-reproductive linyphiids. Among the web-dependent linyphiine species *Bathyphantes gracilis* and *Tenuiphantes tenuis*, remains of the latter were more prevelant in the beetles' guts. *T. tenuis* construct their webs nearer the ground, thus supporting the hypothesis that proximity corresponded to predation. However, among two itinerant hunters, *Erigone* spp. and *Pachygnatha degeeri*, vertical niche separation did not predict predation rates by carabids.

3.1. Introduction

3.1.1. Conservation Biological Control

The direct and indirect environmental costs of intensive farming practices (Stoate *et al.* 2001, Robinson & Sunderland 2002, Benton 2007), and economic value of ecosystem services provided by biodiversity (Costanza *et al.* 1997, Losey & Vaughan 2006, Godfray *et al.* 2010), are becoming increasingly apparent. A major component of more sustainable integrated pest management regimes is conservation biological control (section 1.3) (Risch *et al.* 1983, Bell *et al.* 2002, Griffiths *et al.* 2008, Tscharntke *et al.* 2008). However, increased numbers and diversity of natural enemies do not necessarily translate into improved pest suppression. For example, landscape heterogeneity is usually positively correlated with abundance and diversity of natural enemies (Sunderland & Samu 2000, Bianchi *et al.* 2006, Griffiths *et al.* 2007, Macfadyen *et al.* 2009), but these factors are only associated with increased pest suppression in a minority of cases (Bianchi *et al.* 2006). The mechanisms governing such apparently counter-intuitive findings can be elucidated by examining the interactions between arthropod predators, many of which are trophic generalists.

While polyphagous arthropods can perform a valuable role as beneficial predators in agroecosystems (Symondson *et al.* 2002a), they also have the potential to disrupt biological control by interfering with and consuming one another (Polis & Strong 1996, Daugherty *et al.* 2007, Holt & Huxel 2007). Intraguild predation (IGP) is a form of omnivory where predators (or parasitoids) that share a food resource also engage in trophic interactions (predation or parasitism) with one another (Polis *et al.* 1989, Polis & Holt 1992), and is found widely across food webs (Arim & Marquet 2004). Intraguild interference that results in mortality is thought to be particularly significant among aphidophagous terrestrial arthropods, due to the tendency of their prey to aggregate both in time and space (Snyder & Wise 1999, Lucas 2005).

The ability of a predator assemblage to suppress a population of herbivores depends on its diversity and the environmental context (Straub *et al.* 2008). Studies of emergent predator effects are usually based on a comparison between the feeding rates of a predator species alone and in combination, the null expectation

being that such assemblages will reduce the prey population to the same degree as the additive effects of their constituent species (Sih *et al.* 1998, Schmitz 2007). Mesocosm experiments show that more diverse predator assemblages may simply be more likely to include predator species with higher per capita rates of herbivore consumption, thus more effectively suppressing their shared resource (Straub & Snyder 2006). Increasing predator diversity may, however, lead to reduced herbivore suppression (Rosenheim *et al.* 1993, Finke & Denno 2004), especially where intraguild predators constitute a greater proportion of the predators than strict predators of herbivores (Finke & Denno 2005). The magnitude of herbivore suppression by an assemblage of predators will vary over time (Snyder & Wise 2001) and between herbivorous pest species where more than one is included in the experiment (Wilby *et al.* 2005). Increased predator diversity may also benefit herbivore suppression via positive synergies. Losey & Denno (1998, 1999), for example, suggested that aphids drop from the foliage to avoid predation by coccinellids, thereby increasing their exposure to (mainly carabid) beetles on the ground. Sigsgaard *et al.* (2007) found that the addition to a predator assemblage of generalists whose temporal niches and preference for different life-stages of the pest were complementary, also decreased pest numbers. In a few cases, such complementarity has been shown to correlate with increased crop yield (Cardinale *et al.* 2003, Lang 2003, Snyder *et al.* 2006).

Winter wheat, *Triticum aestivum*, suffers from a number of herbivorous pests, including the grain aphids, *Sitobion avenae, Rhapalosiphum padi* and *Metopolophium dirhodum* (see also section 4.1.1). Aphids can affect crop yield directly as phloem feeders (Larsson 2005, Goggin 2007), and as vectors of major disease such as barley yellow dwarf virus (Fiebig *et al.* 2004). Predator exclusion experiments (Schmidt *et al.* 2003, Holland *et al.* 2008) have shown that overall, aerial aphidophages (principally coccinnelid beetles, true bugs and parasitoid wasps) are superior to ground-dwelling generalist predators (mostly carabid beetles and spiders) at reducing aphid numbers, but in combination, these guilds reduced aphid growth to a greater degree than the sum of either guild alone i.e. the combined effect of the guilds was synergistic. However, many of these aerial natural enemies are more stenophagous, so their numerical response is dependent on aphid numbers, providing an opportunity for aphid populations to establish early in the season (Snyder & Ives 2001, 2003, Symondson *et al.* 2002a). Sustained on

alternative prey (Symondson *et al.* 2000, Agustí *et al.* 2003, Sigsgaard 2007), generalist predator assemblages possess the potential to attack such nascent aphid populations (Harwood *et al.* 2004), causing delays or reductions in population peaks (Edwards *et al.* 1979, Chiverton 1986, Chang & Kareiva 1999, Birkhofer *et al.* 2008).

3.1.2. Study system

Spiders and beetles are the most numerous epigeal arthropod predators in wheat, and these groups are dominated by a small number of species (Sunderland *et al.* 1986, Bell *et al.* 2002, Samu & Szinetár 2002, Gavish-Regev 2008, Tscharntke *et al.* 2008). In Northern Europe, two families of beetles, the Carabidae and the Staphylinidae, predominate (Luff 1978), while the arachnofauna, in terms of numbers, consists principally of members of the Family Linyphiidae. *Bathyphantes gracilis*, *Tenuiphantes tenuis*, *Erigone atra* and *E. dentipalpis* are particularly common (Cocquempot & Chambon 1990, Sunderland & Topping 1993, Samu *et al.* 1996a, Feber *et al.* 1998, Schmidt & Tscharntke 2005). Despite often intensive intraspecific competition, these spiders succeed in efficiently exploiting microhabitats rich in their preferred prey (Harwood *et al.* 2001, 2003, 2004). Linyphiids are essentially generalist insectivores, whose diet consists primarily of diptera, collembola and aphids (Sunderland *et al.* 1987, Janssens & DeClercq 1990). Another key species, *Pachygnatha degeeri* (Araneae; Tetragnathiidae), though less abundant and taxonomically distinct from most common arable spiders, which is nevertheless functionally similar to the linyphiids, displaying high rates of aphid predation (Harwood *et al.* 2005). The spatial niches of all the above species are vertically stratified (Sunderland *et al.* 1986) with *Erigone* spp. preferring to build their webs in small depressions in the ground, while *B. gracilis* and *T. tenuis* attach their webs to the vegetation approximately 45 mm and 36 mm above the ground respectively (Sunderland *et al.* 1986). Adult *P. degeeri* do not spin webs, but are found in the foliage during the night (Madsen *et al.* 2004) and on the ground during the day (Roberts 1996). All five spider species are known to consume *S. avenae* (Sunderland *et al.* 1987, Janssens & DeClercq 1990, Harwood *et al.* 2004, 2005).

The carabid beetle *Pterostichus melanarius* (Coleoptera; Carabidae) is a common, abundant, generalist predator, known to feed on a wide range of prey including insects, molluscs and earthworms (Sunderland 1975, Sunderland *et al.* 1987, Symondson *et al.* 2000, 2002b, Harper *et al.* 2005, King *et al.* 2010b). Most of its lifecycle is subterranean (Sunderland 1987, Thomas *et al.* 2008), where, as a larva, it hunts invertebrates such as slugs (Thomas *et al.* 2009), while adults hunt and scavenge mainly on the surface (but see Snyder & Ives 2001). The beetle is known to be a predator of aphids (Sunderland & Vickerman 1980, Sunderland *et al.* 1987, Harper *et al.* 2005, 2006). Semi-field experiments suggest that *P. melanarius* is also an intraguild predator of smaller carabids, staphylinids (Prasad & Snyder 2004, 2006) and parasitoids (Snyder & Ives 2001) and has been observed to consume erigonid spiders and lacewing larvae in petri dish experiments (Dinter 1998) (see also section 5.1).

Adult *P. melanarius* are outside the prey size range of most of the spiders found in Northern European wheat, while the subterranean larvae are inaccessible to them. Intraguild predation between carabids and linyphiids is therefore assumed to be unidirectional (asymmetrical) (Polis *et al.* 1989).

3.2. Aims and objectives

Here, attempts were made to identify whether *Pterostichus melanarius* is an intraguild predator of aphidophagous spider species using polymerase chain reaction (PCR) (section 2.3, 2.4). Species- and genus-specific primers were used to screen the gut contents of a large population of beetles. It was expected that *P. melanarius*, as a generalist, would consume multiple spider species, but that predation would be non-random. The hypothesis was tested that disproportionately higher numbers of beetles would test positive for those spider species whose temporal and spatial niches overlapped with *P. melanarius* to the greatest extent. The two components of niche in question were diel activity-density (Alderweireldt 1989, 1994a, Chapman & Armstrong 1997, Chapman *et al.* 1999) and vertical spatial proximity to the soil surface (i.e. the spatial nich of *P. melanarius*) (Sunderland *et al.* 1986). In total, three different models were employed to produce null estimates of the numbers of beetles whose guts likely to contain spider remains. The models included the total available prey, with ('Naïve ALL') and without ('Decay ALL') compensation for the bias in prey detection rates, and the

total adult prey with compensation for bias in detectability decay rates ('Decay ADULT'). In testing this hypothesis, the aim was to measure the extent of negative interactions within the aphid generalist predator community, and identify which natural enemy species can coexist with the least antagonism.

3.3. Methods

3.3.1. Sample collection

Samples of ground beetles, spiders and aphids were collected in 2006, from a field of winter wheat (Highfield 4.98 ha. 51.803N, 0.364W) at Rothamsted Research, UK. At each sampling point beetles were pitfall trapped, small arthropods were collected using Vortis sampler, subsequent hand search of the litter and aphids from 20 randomly-chosen wheat heads, the latter of which were removed by hand. A total of 622 *Pterostichus melanarius*, were collected using small (9 cm Ø) pitfall traps laid overnight for 12 h. The maximum time beetles could remain in the pitfall traps was therefore 12 h. Beetles were put on ice in the field and transferred to separate micro centrifuge tubes before being frozen at -80°C within 2 h of collection. Small arthropods, including spiders, were collected by Vortis sampler (Burkhard Ltd, Rickmansworth, UK) from quadrats, a process that was followed by a hand-search of the top layer of soil. At each point, samples were taken at three stages: flowering (week beginning 12 June: Zadoks scale 69 - 70); milky or mealy ripe ears (week beginning 10 July: Zadoks scale 73 - 85) and at harvest (week beginning 31 July: Zadoks scale 90 - 92). There were 80 sample points, 16 m apart, across the field, but for the purposes of this analysis, data were pooled for each invertebrate species on each date because details of the provenence of the beetles was lost.

3.3.2. Sample preparation and DNA extraction

DNA was extracted from beetle foreguts according to the 'animal tissue' protocol of the Qiagen DNeasy tissue kit (Qiagen Ltd, Crawley, UK), from non-target prey using the 'solid tissue' protocol from a Puregene extraction kit (Gentra Systems Inc. Minneapolis, PA, USA) and from spider juveniles using a chelex-based method (Bio-Rad Ltd, Hemel Hempstead, UK). For full details see section 2.2.2.

3.3.3. PCR screening

Gut content analysis of *P. melanarius* was carried out in duplicated singleplex PCR reactions using the primers detailed in Table 3.1 to attempt amplification of COI mitochondrial DNA of the following: a 271 bp fragment of *Bathyphantes gracilis*, a 244 bp fragment of *Erigone* spp. (targetting two species *E. atra* and *E. dentipalpis*), a 318 bp fragment of *Pachygnatha degeeri* and a 145 bp fragment of *T. tenuis*.

Adult spiders were identified morphologically using the key of Roberts (1996). However, it is not always possible to identify juvenile spider to species level based on morphology when they have been subjected to damage from suction sampling. A multiplex PCR reaction containing primers for *B. gracilis*, *Erigone* spp. (*E. atra/E. dentipalpis*) and *T. tenuis* was used to simultaneously assign linyphiid juveniles to species (detailed section 2.4.2, Appendix V). DNA extracts were first tested with this multiplex PCR. Those testing negative at this stage were subsequently screened with *Pachygnatha degeeri*-specific primers. If a sample was negative for both assays, they were screened for a 710 bp fragment of the COI gene using general primers (Folmer *et al.* 1994) to check that DNA extraction had been successful. All the PCRs for identifying juvenile species were carried out in 8 µL reactions, each of which comprised of 4 µL of Qiagen Multiplex PCR Mastermix, 0.08 µL Q-Solution (Qiagen), 0.08 µL Bovine Serum Albumin (New England Biolabs, Ipswich, MA,USA), 0.8 µL template DNA and 3.4 µL H_2O in a Bio-Rad DNA Engine Peltier thermal cycler (Bio-Rad, Ltd, Hemel Hempstead, UK) and subjected to the following cycling parameters: initial denaturing temperature of 94°C for 15 min, then for 35 cycles of: denaturation at 94°C for 90 s, annealing at 60°C for 45 s, extension at 94°C for 45 s, then a final extension of 72°C for 10 min, after which products were stored at 10°C. Cross-amplification testing of the multiplex reaction was carried out on the same panel of species as mentioned above (Appendix V).

Conventionally, only those predators that test negative for prey remains are re-screened. However, to assess the consistency of the PCR assays, each screening was duplicated whether or not it proved negative on the first attempt, allowing the effects of this increase in screening effort to be measured.

Target species	Primer name	Primer sequence 5'- 3'	Fragment size (bp)	PCR conditions					
				Ta (°C)	[$MgCl_2$] (μM)[1]	[dNTPs] (μM)[1]	[Primer] (μM)[2]	Taq (U)[1]	BSA (μM)
Bathyphantes gracilis	B_grac-C1-80F*†	GGA GAT GAC CAT TTA TAT AAT GTC	271	61	3	002	02	06	01
	B_grac-C1-350R*†	AAT GCC CTT CTA AAG AAG CTA A							
Tenuiphantes tenuis	T_ten-C1-306F*†	GCT TCT TTG GAA GGT CAT GCA	145	62	3	02	02	06	01
	T_ten-C1-450R*†	CTT TTT CTA TAG ATA CCC CAT ACC C							
Pachygnatha degeeri	P_degeeri_C1-277F*	GGC TAC TTC CCC CTT CGT TGT TC	318	64	3	002	015	06	01
	P_degeeri_C1-596R*	TAG CAT TGT AAT AGC CCC CGC T							
Erigone atra and E. dentipalpis	Erigone_sp_C1-146F*	GAA CAA TTT ATC CTC CTC TAG C	244	62	35	002	02	06	01
	Erigone_sp_C1-389Rb*	GTG ATA GCT CCT GCA AGC AC							
	Erigone_sp_C1-185F†	CTG GTA GTT CTG TTG ATT TTG CA	205	n/a	n/a	n/a	n/a	n/a	n/a
	Erigone_sp_C1-389Rb†	GTG ATA GCT CCT GCA AGC AC							

Table 3.1. Primer sequences, predicted fragment sizes, optimal reagent concentrations and annealing temperatures for detection of each target species. * primers used in singleplex reactions. †primers used in multiplex reactions. [1]Supplied by Invitrogen, [2]supplied by Eurofins MWG Operon (Ebersberg, Germany), [3]supplied by New England Biolabs (Ipswich, MA,USA).

3.3.4. Feeding trials

Feeding trials were undertaken for each of the four prey amplicons (see section 2.5 for details). Each predator, like the field-caught gut samples, was screened twice regardless of whether the first attempt at amplification was successful.

3.3.5 Statistical analysis

3.3.5.1 Feeding trials

It is important to make some quantitative measure of the rate at which the detectability of a given fragment in a given predator will decay over time. Cumulative proportions of beetles testing positive following two screenings for each spider amplicon at each time period were calculated and subjected to binomial regression analysis (with a logit link function) using R version 2.10.1 (R Core Development Team 2010). The regression equation was then rearranged to estimate the time, post-feeding, at which a given proportion of predators would likely test positive, using the 'MASS' package in R (Venables & Ripley 2002). The convention is to estimate the T_{50}, but for the Monte Carlo models employed to test the present hypothesis, the T_{90} of the slowest-decaying amplicon was used as a proxy for the time it took for the amplicon to become undetectable. Additionally, the differences between decay rates were assessed by modelling the cumulative proportions of beetles testing positive for all four spider fragments in a single general linear model, followed by Tukey's pairwise comparisons using the 'multcomp' R package (Hothorn *et al.* 2008).

3.3.5.2. Analysis of predation using Monte Carlo models

Monte Carlo randomisation models were employed to test whether the predator *P. melanarius* consumed spider species at random, in proportion to their abundance in the field. The Monte Carlo approach of Agustí *et al.* (2003) and King *et al.* (2010b) was initially used, in which the structure of the original data is retained in the model (i.e. the number of beetles and primer positive results per beetle), but the identities of the detections within each beetle are allocated randomly: the probability of a particular prey being 'eaten' is proportional to its numerical abundance in the field. Following 20,000 iterations, the model produced 'expected' consumption rates. A two-tailed test of whether observed rates fell outside defined confidence intervals (either 95%, 99% or 99.9%) indicated the extent to which

predation deviated significantly from the null, random-foraging model (Manly 1997a, b). Simulations were run in R version 2.10.1 (R Development Core Team 2010).

Adjusting for decay rates is difficult, as this is a time-dependent process in which the precise feeding times are unknown. In a second, revised Monte Carlo model, we incorporated typical activity patterns for *P. melanarius* which, on the assumption that feeding rates are proportional to activity, represented a probability density function for feeding times. The species' circadian activity pattern, estimated from the 'open vegetation' results from Fig. 1 in Chapman *et al.* (1999) (which was considered most similar to winter wheat), followed a normal distribution (with a peak at 10:15pm and a 3.2 h SD around this mean). The time window for feeding was bounded by the time of capture and the T_{90} of the slowest-decaying fragment, i.e. the point at which the detection probability from the logistic regressions was 0.1 (*T. tenuis* after 91.2 h). Varying this upper limit (for probabilities of 0.05 – 0.20) had no effect on the results.

The simulation model permuted the predation events that were detected using the PCR assay. The resulting decay rate simulation ran in the following steps for each primer positive result: i) a prey item was selected at random ('eaten') based on the relative prey densities (as in Agustí *et al.* 2003, King *et al.* 2010b), ii) a feeding time was drawn at random from the activity pattern, iii) the detection probability, p_i, for the prey item was calculated from the appropriate feeding trial regression, based on the elapsed time between feeding and freezing the beetle, and iv) a uniform random number, r, between 0 and 1 was drawn, to select whether the 'eaten' prey item was detected (where $r \geq p_i$). These stages were repeated until a fragment was successfully detected. This revised model was again run through 20,000 iterations.

Three discrete models were run, (1) including all available prey (adult and juvenile spiders), but without correction for decay and predator diel activity (Naïve ALL), (2) including all spiders and accounting for amplicon decay rates and the beetle activity (Decay ALL), and (3) a model taking account of the amplicon decay rates, but including only adult spiders, i.e. assuming juveniles were not a part of the beetles' diet (Decay ADULT).

3.3.5.3 Diel overlap

Diel overlap was estimated by producing indices of Pianka's niche overlap (Pianka 1973) between *P. melanarius* and the prey using data collected from agricultural ecosystems in the literature (Alderwiereldt 1989, 1994a, Chapman & Armstrong 1997, Chapman *et al.* 1999). Data was analysed using ECOSIM v 7.72 (Gotelli & Entsminger 2004) (Kesey-Bear and Acquired Intelligence, Inc, Jericho, VT, USA)) using default 'Niche overlap' settings.

3.3.5.4 PCR duplication

A comparison of the consistency of the PCR screenings was undertaken by means of a general linear mixed effects model with the proportion of predators testing positive as the response variable, the number of cumulative screenings a fixed explanatory factor and prey species as a random explanatory factor. Additionally, a generalised linear model was employed to test how the proportion of predator assays that agreed between the first and second screening responded to the proportion of cumulative positives.

3.4. Results

3.4.1. Spider populations

Sampled populations of spiders are detailed in Table 3.2. As expected, it was found that the most common arable linyphiids were numerically dominant in this study: a combination of *Erigone* spp., *Tenuiphantes tenuis* and *Bathyphantes gracilis* made up 70.9% and *Pachygnatha degeeri* 4.4% of the total spider population. A small number of spiders (n = 15 out of 2702*)* generated more than one species-specific band. This was probably due to low levels of IGP among juvenile spiders, as contamination between extractions was controlled for using negative control extractions in each batch (see section 2.2.2).

Species		June	July	August
Tenuiphantes tenuis	Adult	80 (0.24)	95 (0.08)	129 (0.11)
	Juvenile	57 (0.17)	432 (0.35)	491 (0.43)[7]
	Total	137 (0.42)	527 (0.43)	620 (0.54)[7]
Bathyphantes gracilis	Adult	49 (0.15)	91 (0.07)	3 (<0.01)
	Juvenile	30 (0.09)	197 (0.16)	71 (0.06)[7]
	Total	79 (0.24)	288 (0.23)	107 (0.09)[7]
Erigone spp.	Adult	12 (0.04)	31 (0.03)	7 (0.01)
	Juvenile	11 (0.03)	53 (0.04)	44 (0.04)[7]
	Total	23 (0.07)	84 (0.07)	51 (0.04)[7]
Pachygnatha degeeri	Adult	5 (0.02)	9 (0.01)	13 (0.01)
	Juvenile	17 (0.05)	46 (0.04)	6 (0.01)[7]
	Total	22 (0.07)	55 (0.04)	19 (0.02)[7]
Oedothorax spp.		4 (0.01)	12 (0.01)	10 (0.01)
Other tetragnathids		4 (0.01)	5 (<0.01)	18 (0.02)
Xysticus spp.		3 (0.15)	21 (0.02)	37 (0.03)
Lycosidae		5 (0.02)	8 (0.01)	81 (0.07)
Others/Unknown		51 (0.16)	229 (0.19)	187 (0.16)
Mixed		0 (0.00)	7 (0.01)	8 (0.01)
Grand total		328	1236	1138

Table 3.1. Total numbers of adult and juvenile spiders collected across the 80 sampling points on each date numbers in parantheses indicate proportions of grand total. *Erigone* spp. spiders consisted of both *Erigone atra* and *E. dentipalpis*. A number of individuals could not be extracted[7]

3.4.2. Decay rates calculated from feeding trials

Although we did not use T_{50}s for the Monte Carlo simulations, they are given here so that our primers can be compared with those used in previous studies (Fig. 3.1). T_{50}s for *Pterostichus melanarius* eating spiders were 29.0 h for the 271 bp

[7] DNA extraction was impossible for ~10% of the juvenile spiders sampled in August (samples dried up in storage tubes), and so total numbers for these were extrapolated in proportion to successfully identified juveniles (from August only). A few spiders (0.8% of total juveniles) that tested positive for more than one species (possibly caused by intraguild predation or contamination during sampling) were classified as unknown, and hence excluded from the Monte Carlo analyses.

fragment of *B. gracilis* DNA, 42.6 h for a 145 bp fragment of *T. tenuis*, 19.7 h for a 244 bp fragment of *Erigone* spp. and 29.6 h for a 318 bp fragment of *P. degeeri*. A general linear model showed the probability of successfully amplifying a fragment of DNA from this limited range of spiders was not explained by fragment size and no pair of assays differed significantly from any other (Table 3.3). Predictably, however, amplification success was significantly related to digestion time (GLM, $t = 6.897$, $P < 0.001$).

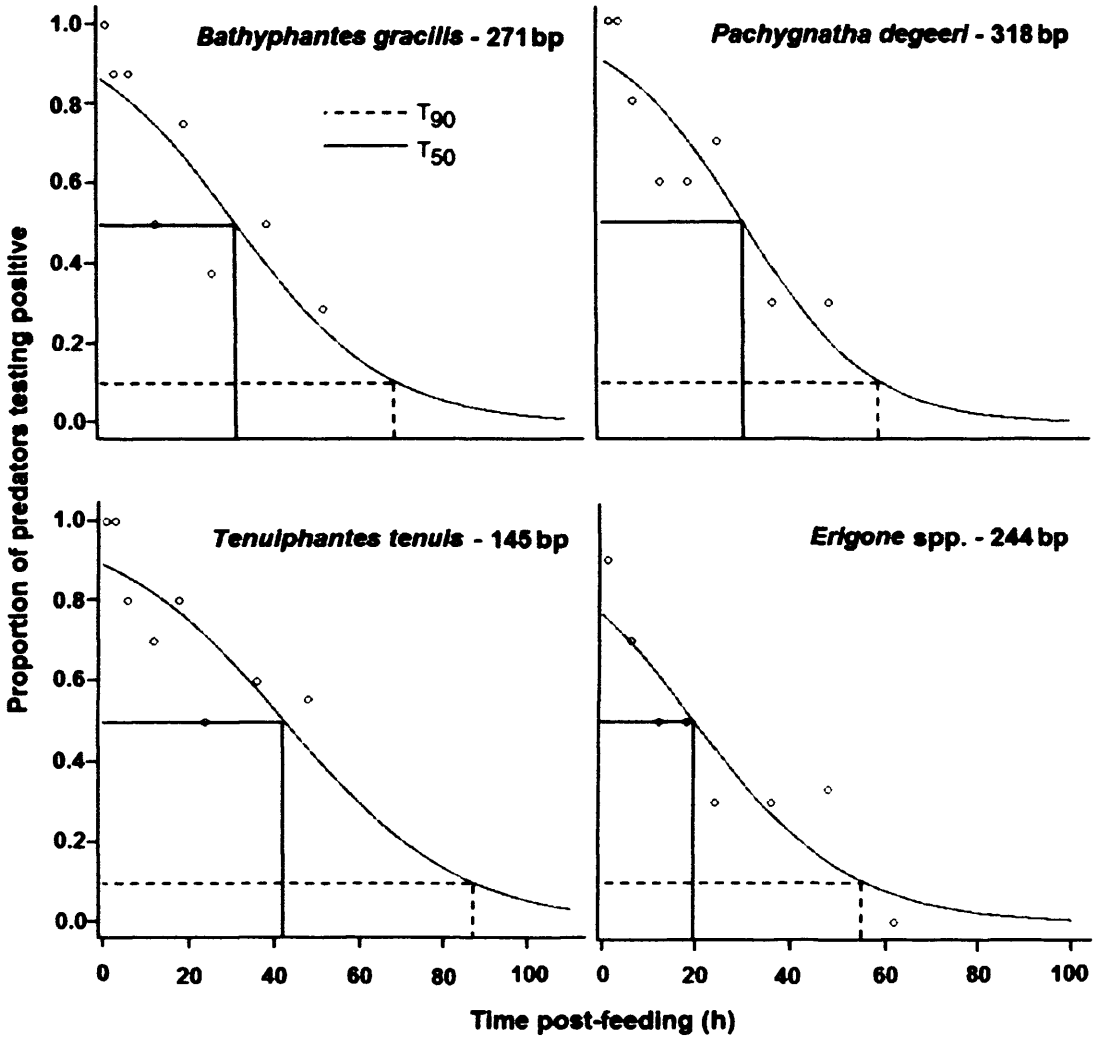


Fig. 3.1. Decay rates of target fragments of COI mtDNA in the guts of *Pterostichus melanarius*. T_{50} (median detection) and T_{90} periods were estimated by fitting data from the feeding trials to a binomial regression model. Such a model ensures that for any value of the predictor (time post-feeding), the response (proportion of successful amplification attempts) is bounded between 0 > 1, the sample size data was retained (8 - 10 beetles per time period) and non-normal error distributions were accounted for (Crawley 2007).

Species	*B. gracilis*	*Erigone* spp	*P. degeeri*
Erigone spp.	P = 0.514		
P. degeeri	P = 0.996	P = 0.33	
T. tenuis	P = 0.803	P = 0.087	P = 0.885

Table 3.3. Pairwise analysis of each fragments' decay rates based on the cumulative number of positives from two screenings, using Tukey's tests.

3.4.3. DNA detection rates, sampled population sizes and predator prey breadth

DNA from every spider species for which primers were available was found in the guts of *P. melanarius* during every month of sampling. Overall, predation on spiders was lowest in June, when beetle and spider numbers were low. However, the proportion of beetles testing positive for spider DNA was highest in June, when 43.6% (61 of 140 beetles) were positive, compared with 35.3% in July (96 out of 272) and 33.3% in August (70 of 210). However, no month was significantly different from any other ($\chi^2_2 = 4.0999$, P = 0.1287). Fig. 3.2 shows the number of beetles testing positive for each spider species in the form of single predator food webs. The topology of the webs was very similar over the three months, suggesting predation was mainly on *T. tenuis*, with little consumption of other species. The number of beetles testing positive for more than one target spider species simultaneously (DNA from more than one spider species detected in a beetle) was low, with six (4.2%) in June (one of which scored positive for 3 species), five (1.8%) in July, and 2 (2.9%) in August.

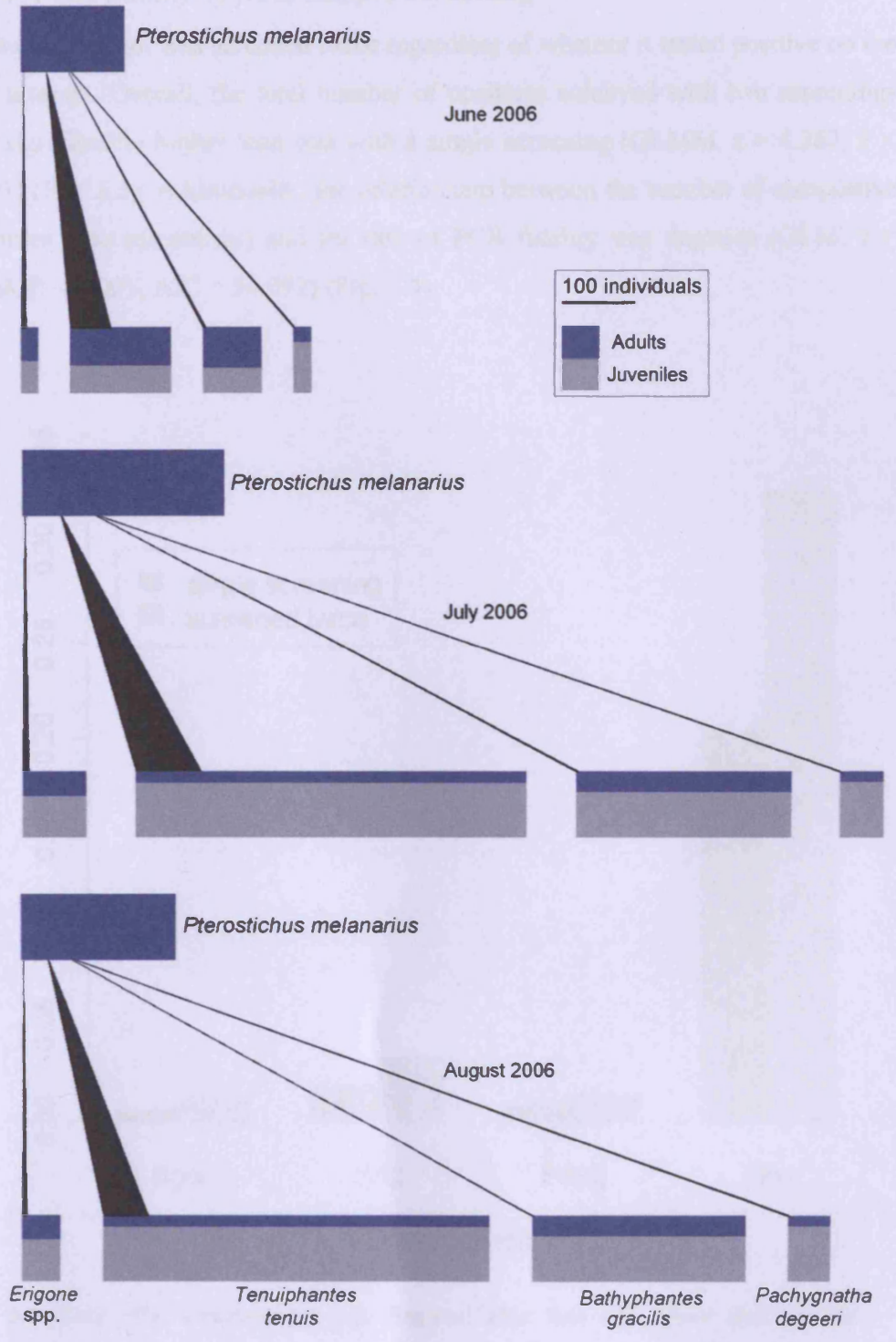


Fig. 3.2. Bipartite food webs showing the proportions of *Pterostichus melanarius* testing positive for each spider species/group in each month. The width of each block represents the abundance of each species (reflecting activity-density in the case of *P. melanarius*, absolute density for spiders) and the width of the lower part of the arrows the number of beetles testing positive for each species of spider. The same scale is used for beetles and spiders. All beetles are adult.

3.4.4. PCR fidelity of field sample screening

Each predator gut was screened twice regardless of whether it tested positive on the first attempt. Overall, the total number of positives achieved with two screenings was significantly higher than that with a single screening (GLMM, $z = 4.382$, $P < 0.001$) (Fig. 3.3). Additionally, the relationship between the number of cumulative positives (two screenings) and the rate of PCR fidelity was negative (GLM, $z = 5.338$, $P < 0.001$, AIC = 34.092) (Fig. 3.4).

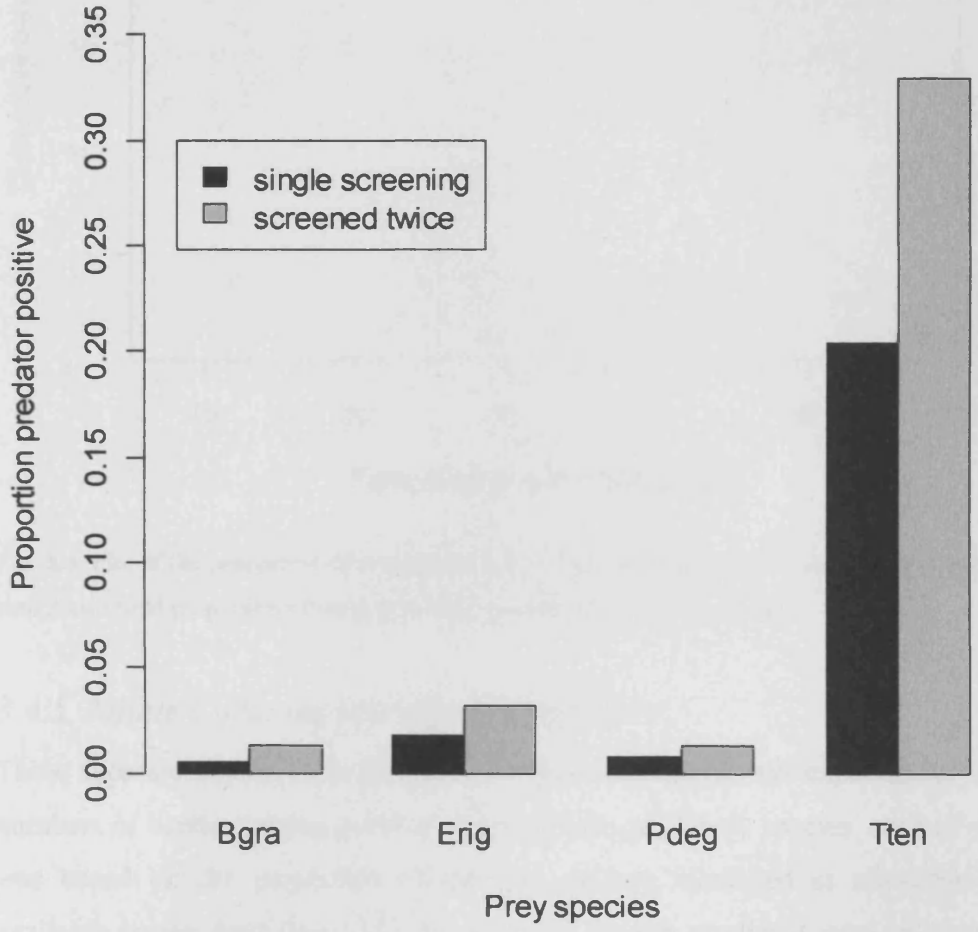


Fig. 3.3. Total PCR successes for each fragment after first and second attempts. Bgra = *Bathyphantes gracilis*, Erig = *Erigone* spp., Pdeg = *Pachygnatha degeeri*, Tten = *Tenuiphantes tenuis*.

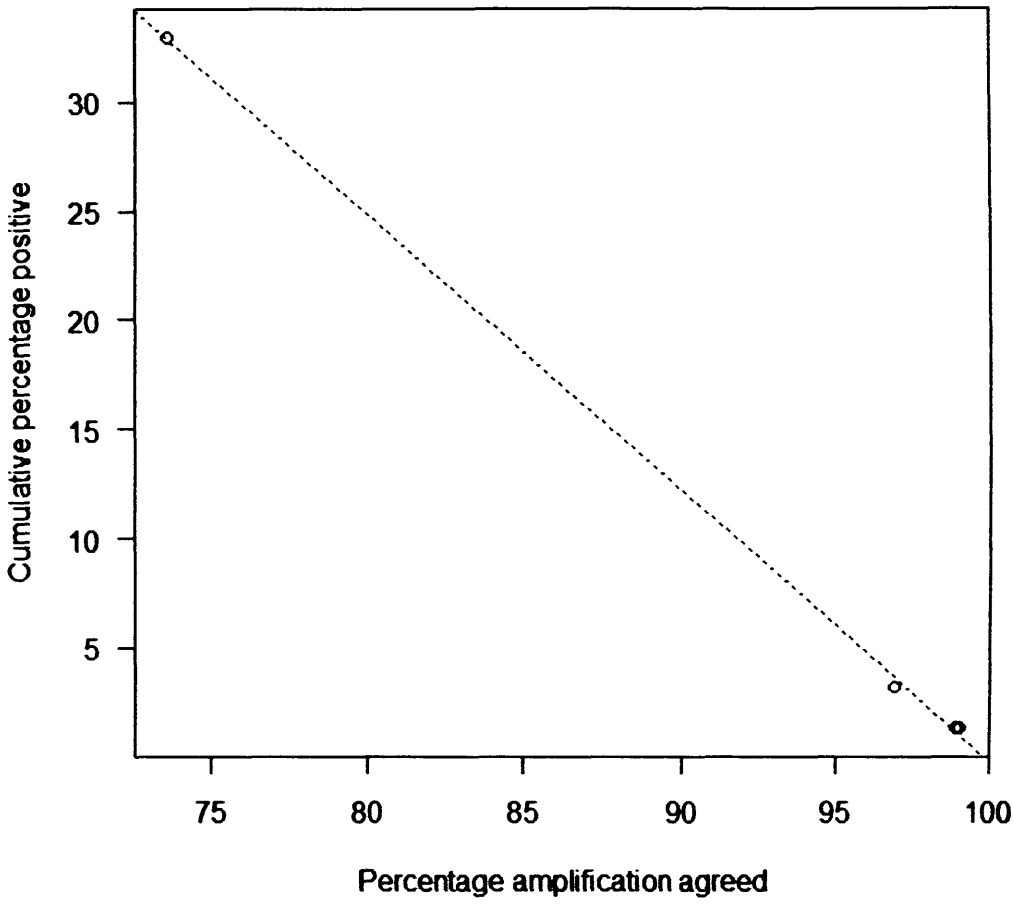


Fig. 3.4. Plot of the proportion of samples for which both replicates of screening agreed against the cumulative total proportion of samples testing positive after two screenings.

3.4.5. Monte Carlo models of prey preference

Three separate Monte Carlo models were run to produce null expectations of the numbers of beetles testing positive for every intraguild prey species, each of which was based on the proportion of the four spiders identified as intraguild prey available in the field (Fig. 3.5). Each of the models predicted rates of predators testing positive for each amplicon (when discussed in the context of the Monte Carlo models, this proxy measure is hereafter referred to as 'predation' for brevity), were then compared to the observed rates of 'predation'.

Where both adults and juvenile spiders were included in potential prey numbers, but no correction was made for the differences in detectability of the prey amplicons ('Naïve ALL'), there were no difference found between observed and expected levels of predation for the two itinerant spiders, *P. degeeri* and *Erigone*

spp. for any month, while differences for the web-dependent linyphiines *T. tenuis* and *B. gracilis* were apparent for all months. *B. gracilis* was detected significantly less frequently than expected, while *T. tenuis* was detected significantly more often than expected.

The incorporation of differences in amplicon detection ('Decay ALL') resulted in predicted levels of predation identical to the 'Naïve ALL' model with just one difference: the number of beetles predicted to contain remains of *T. tenuis* in August was not significantly different from the observed numbers. However, the level of significance fell from 99.9% to 95% for both linyphiine species in all months. Differences between observed and expected results remained non-significant for *Erigone* spp. and *P. degeeri* for the 'Decay ALL' model.

In the third model ('Decay ADULTS'), juvenile spiders were removed from the counts from which the prey proportions were drawn, but beetle diel activity and fragment decay rates were included. The differences between observed and expected rates of predation retained a similar pattern to the other models. Among the linyphiines, the number of beetles testing positive for *B. gracilis* remained significantly lower than expected, while significantly more beetles contained *T. tenuis* DNA. For the itinerant spiders, fragments of *Erigone* spp. were still amplified in the numbers predicted except for August, when significantly more fragments (at the 95% CI) were amplified for this species than this model predicted. However, for *P. degeeri*, significantly fewer beetle guts contained fragments of this species than predicted in all months (significant at 99.9% CI).

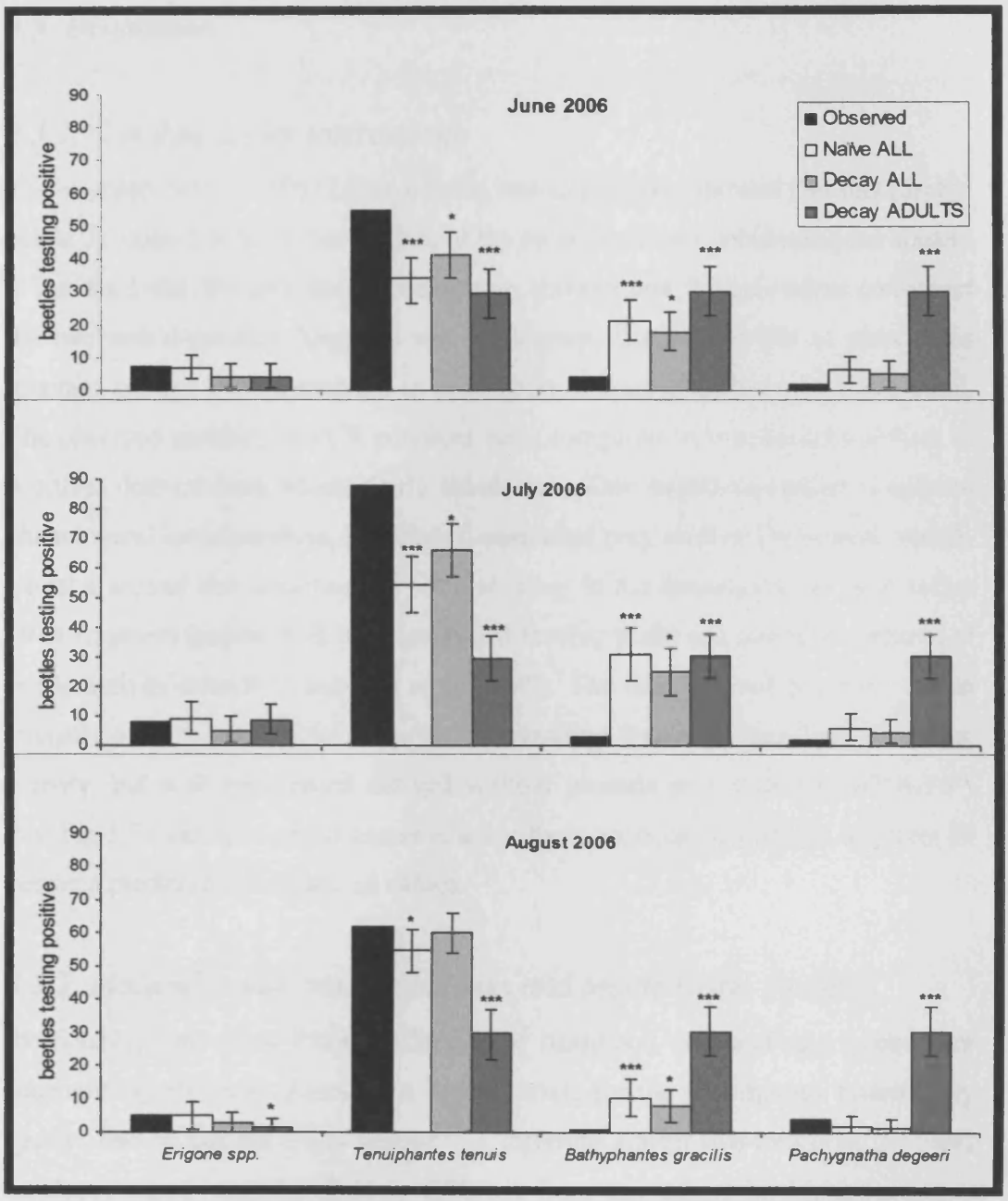


Fig. 3.5. Numbers of *Pterostichus melanarius* testing positive for spider prey (black bars) compared with expected predation rates generated by Monte Carlo randomisation models (20,000 iterations). Three simulations were run: (1) 'Naïve ALL' (white bars) including just relative prey availability (2) 'Decay ALL' (light grey bars) model incorporated, in addition to relative prey availability, species-specific prey detection periods and relates circadian activity-density of the predator to the probable times at which the beetles were captured. (3) 'Decay ADULTS' (dark grey bars), incorporated decay rates and circadian activity but only included adults of the intraguild prey species. Error bars represent 99% confidence intervals, while the asterisks denote the probabilities that the predicted levels of beetles testing positive fall within the tails of the distributions of random permutations of the data (two-tailed test: *$\alpha = 0.05$; $P < 0.025$ or $P > 0.975$, **$\alpha = 0.01$; $P < 0.005$ or $P > 0.995$, ***$\alpha = 0.001$; $P < 0.0005$ or $P > 0.9995$).

3.5. Discussion

3.5.1. Carabid-spider interactions

Post-mortem analysis of 622 *Pterostichus melanarius* guts showed that this carabid beetle consumed at least four species of the most numerous aphidophagous spiders at our field site. Broadly these comparisons showed that *P. melanarius* consumed the two web-dependent linyphiid species disproportionately, while in most cases itinerant spiders were consumed in proportion to their abundance on the ground. The observed numbers of PCR positives were compared with expected numbers of positives derived from Monte Carlo simulations. One model was based simply on phenological co-occurrence, i.e. relative numerical prey availability in each month, whilst a second also incorporated rates of decay in the detectability of each target DNA fragment (established from controlled feeding trials) and circadian patterns of beetle activity-density (Chapman *et al.* 1999). The latter proved the most robust comparison (see below), so a model incorporating fragment decay and circadian activity, but with prey ratios derived without juvenile prey ('Decay ADULTS') (see Fig 3.5) was also run to assess whether their omission produced a different fit between predicted and observed values.

3.5.2. Molecular detection techniques and Monte Carlo models

Predictably, time since feeding affected the likelihood of detecting a given prey fragment significantly. Among the feeding trials for the four spiders tested, prey species had no overall effect despite T_{50s} differing almost two-fold (Fig. 3.1) and no decay curve was significantly different from any other. In this experiment, therefore, it was unsurprising that incorporation of rates of decay in detectability into the Monte Carlo null models did not produce appreciable differences in the statistical comparisons of each null model with the observed values. However, the predicted predation rates of the model incorporating decay rates and activity periods ('Decay ALL') were more closely aligned to the pattern of observed predation than the simple model, suggesting that the incorporation of these parameters created a more robust test of whether feeding was non-random.

As in all such molecular analyses of invertebrate gut contents, we cannot be sure whether scavenging was a significant factor in this study (Foltan *et al.* 2005). However, the observed patterns of primarily density-dependent consumption by *P. melanarius* on itinerant species, and non-random consumption of more web-dependent species, suggests that active predation occurred, as opposed to scavenging of dead individuals.

Prey detection using PCR has a number of limitations including non-specificity to the sex or life-stage of the prey and the possibility of false positive results as a consequence of secondary predation. Error due to the latter is, however, probably minimal (Sheppard *et al.* 2005), even where detection periods are longer than in the present study (Harwood *et al.* 2001). The concentration of prey remains in each predator may be estimated using quantitative PCR (qPCR), and the precision of such an approach is increased by using multiple target fragments (Troedsson *et al.* 2009). However, distinguishing between the predator having consumed a small meal recently, or a large meal less recently remains intractable, compounded by differences in size between adults and juveniles, and between sexes in sexually dimorphic species such as *Bathyphantes gracilis* and *Tenuiphantes tenuis*, in which females are larger than male (Harwood *et al.* 2003). Thus, these results assume only one prey species was eaten during each predation event to match that which occurred during the feeding trials, resulting in conservative estimates of predation strength (Naranjo & Hagler 2001).

Resampling methods such as the Monte Carlo models employed here avoid problems with distributional assumptions of classical tests, because the reference distribution they use for hypothesis testing is generated from the data themselves, as opposed to some standard distribution (Legendre & Legendre 1998). They are thus suitable for hypothesis testing even when sample sizes are small (Legendre & Legendre 1998). The models employed here, however, only provide a *relative* test of prey choice. In order to gain a more thorough insight into the choice of prey, all the potential prey items should be included in the model, a difficult task for a highly polyphagous predator such as *P. melanarius*.

3.5.3. Predation on linyphiids

Tenuiphantes tenuis suffered the highest rates of consumption, with 33.0% of the beetles testing positive for this species overall, while the lowest overall rate was seen in *B. gracilis* at 1.3% (Fig. 3.2). Beetles positive for *T. tenuis* was significantly higher than the expected 95% confidence intervals estimated by all three Monte Carlo simulations except for one model in one month; the 'Decay ALL' model in August. Rates of predation on *B. gracilis*, a species that builds its webs further from the ground than *T. tenuis*, (~45 mm as opposed to ~36 mm), were consistently lower than the predicted range generated by all simulations in every month (Fig. 3.2). For these two species, whether juveniles were included or not, there was a clear preference for *T. tenuis*, while *B. gracilis* was not favoured. Taken together, these results indicate that consumption was positively associated with vertical proximity to the prey, suggesting that this characteristic of the prey was a contributory factor in the apparency (i.e. the likelihood of encounter rates) of the prey to ground-dwelling predators.

The number of beetles testing positive for the itinerant species was low compared to linyphiines, with *Erigone* spp. present in 3.2% of beetles, while *Pachygnatha degeeri* was found in 1.3% of cases. When these rates were corrected for prey abundance ('Naïve ALL'), they fell within the ranges predicted by resampling for both species in all months. Furthermore, when primer detection rates were also included in the model ('Decay ALL'), incidences of amplicon detection was also within the predicted bounds, suggesting that rates of beetles consuming at least one individual of these species simply reflected the prey species' population densities. There was hence no evidence of differential prey choice. When juveniles were removed from the model ('Decay ADULTS'), the rates of detection of *Erigone* spp. remained within the predicted range in June and July, but were disproportionately high in August. The comparisons between observed detection rates and those predicted by all three models for *Erigone* spp. were therefore contrary to expectations, because as ground based web-builders, they ought to have had the highest encounter rates with *P. melanarius*, and consequently suffered proportionately higher rates of predation than the other spiders once detection rate errors were accounted for.

The diel activity patterns of the predator were included in the 'Decay ALL' and 'Decay ADULT' Monte Carlo models (in which it was assumed that feeding rates were proportional to activity), but the prediction that higher prey DNA detection rates in the guts of predators are positively related to encounter rates between predator and prey also depends on the temporal niche of the intraguild prey. While they preferentially use webs for hunting (Harwood 2004), both *T. tenuis* and *B. gracilis* often spend time on the ground foraging (Harwood *et al.* 2001) and dispersing (Alderwiereldt 1989). A comparison of their circadian activity patterns using Pianka's niche overlap index (P_{ij}, Pianka 1973) may thus provide estimates of encounter rates between the species on the ground and hence the apparency of the prey to the predator. A comparison of pitfall trap catches of *P. melanarius* in open vegetation (Chapman *et al.* 1999) with that of *T. tenuis* and *B. gracilis* (see Alderweireldt 1994a), shows that *P. melanarius* and *T. tenuis* share the highest level of diel overlap ($P_{ij} = 0.970$), whilst the levels of coincidence with activity-density of *B. gracilis* ($P_{ij} = 0.871$) are somewhat lower, suggesting this factor may also plays a role in the preferential consumption of *T. tenuis* over *B. gracilis*.

Ground activity measured by pitfall trapping for *Erigone* spp. (Chapman & Armstrong 1997) also indicates a high degree of temporal overlap with *P. melanarius* ($P_{ij} = 0.912$). Prey choice among surface-dwelling carabids as measured by the proxy of the number of predators testing positive for a given species, is apparently density-dependent (King *et al.* 2010a, Hatteland *et al.* in prep.) and is sometimes dynamically linked in terms of the beetles functional response, to pest prey species (Bohan *et al.* 2000, Winder *et al.* 2001, 2005). It is thus unsurprising that the consumption of *Erigone* spp. also seems to be density-dependent. In relatively sedentary prey species (slugs, worms and aphids), there is a high likelihood of encounters between mobile predators and sedentary prey, and vice versa (Rosenheim & Corbett 2003). It is therefore also possible that the geographically wide-ranging co-occurrence of *Erigone* spp. with *P. melanarius*, combined with predation pressure, might have led to rapid evolution of behaviours that help these spider species to avoid predation by the beetle (Magalhães *et al.* 2005).

Laboratory experiments by Madsen *et al.* (2004) found that adult *P. degeeri* forage at night high in the foliage at the time when *P. melanarius* are most active on the ground (Chapman *et al.* 1999). Juvenile *P. degeeri*, conversely, occupy a spatial niche closer to the ground with little vertical diel movement (Roberts 1996). Thus, it might have been expected that any DNA of this species found in predator guts would, all other things being equal, more likely be remains of juveniles than adults. The finding that observed incidences of *P. degeeri* were significantly lower than expectation (99.9% CI) in the 'Decay ADULT' lends support to this notion because while the removal of juveniles from the model did not result in any changes in significance for the other three spider species, in *P. degeeri* this was the case. PCR limits the robustness of such conclusions, however, because the method cannot distinguish between life stages. The issue could potentially be resolved by raising antibodies specific to a particular life stage for use with enzyme-linked immunosorbent assays (ELISA) (Symondson 2002).

3.5.4. Interpreting PCR positives as predation rates

The method employed produced conservative estimates of predation because it assumed that only one prey item was eaten for each predator that tested positive. Attempting to extrapolate from the number of predators testing positive for a prey species to absolute predation rates of this species is complex. Maximal feeding rates over the duration of the fragment's detectability in the gut may be established in the laboratory (Sopp & Wratten 1986), but such experiments often do not consider alternative prey. The frequency distribution of number of prey consumed by a population of predators in the field is likely to be affected by the predators functional response (Berlow *et al.* 2004), prey toxicity (Bilde & Toft 1997), environmental conditions (Kruse *et al.* 2008), competition and prey switching (Abrams & Ginzburg 2001). Nevertheless, whatever frequency distribution of predation rates is assumed (other than all predators eating one prey item), predation rates will be disproportionately higher where more predators test positive for a given prey species. If the simplifying assumption is made that different prey types have the same frequency distribution, then predation on the more commonly amplified prey species will thus be disproportionately greater. In the present study, the higher rates of detection of *T. tenuis* DNA implies that this species has suffered disproportionately high levels of predation compared to the other spider species.

3.5.5. Consequences of intraguild predation for control of aphids

High rates of intraguild predation on those spider species that exhibit higher aphid consumption rates are more likely to bring about TCs (e.g. Borer *et al.* 2005), increasing the subsequent possibility of lower crop yields. While spiders' functional responses to aphids (i.e. type II) are generally similar across a range of species in the laboratory (Mansour & Heimbach 1993), they are nevertheless dependent on a number of factors unique to functional groups or species. Micryphantid spiders such as *Erigone* spp. consume, on average, ~1 small prey item (usually diptera, aphids or collembola) per day (Nyffeler & Benz 1988, Nyffeler & Sunderland 2003). Little work has been done on the prey choice and predation rates of *P. degeeri*, but the work that has been undertaken suggests that while the species is usually less numerous, individuals are more likely to consume aphids (Harwood *et al.* 2005).

Large intraspecific differences have also been observed in aphid consumption rates based on *T. tenuis* success at securing a web: in a microhabitat rich in the species' favoured prey such as aphids, collembola and diptera, web owning individuals may eat approximately 2.5 times as much aphid prey as non-web owners (Harwood *et al.* 2003). Harwood *et al.* (2004) showed that in *T. tenuis*, the response to aphids was independent of alternative prey (collembola) availability, whilst for *Erigone* spp., predation on aphids was negatively associated with collembola abundance. However, this is contrary to findings from laboratory experiments, in which aphid predation rates of *Erigone* spp. were independent of the density of a model alternative prey (*Drosophila melanogaster*) (see Madsen *et al.* 2004). This latter phenomena may perhaps be explained when the reproductive benefits (i.e. increased offspring) of the favoured collembolan prey of *Erigone* spp., *Isotoma anglicana* (see Agustí *et al.* 2003), are considered, which apparently outweigh those of *D. melanogaster* (see Marcussen *et al.* 1999). Impacts on populations of *P. degeeri* through IGP, however, seem to have a greater potential for pest release. Aphid consumption by *P. degeeri* was also found to be independent of *D. melanogaster* density in laboratory trials (Madsen *et al.* 2004), and quantitative measures of aphid protein remains in the guts of *P. degeeri* suggest that it consumes aphids at disproportionately high levels in comparison to other spiders screened from field samples (Harwood *et al.* 2005).

Predation by carabids on the more common *T. tenuis*, in particular, may have the potential to release aphids from predation by these spiders. Furthermore, it is likely, given more *P. melanarius* contained remains of *T. tenuis*, more *P. melanarius* would have consumed more than a single *T. tenuis* individual. Despite these considerations, the abundance of *T. tenuis*, this 'preferred' intraguild prey of *P. melanarius*, continued to increase across the season (Table 3.2). The degree to which *T. tenuis* numbers may be limited by *P. melanarius* will depend upon their relative densities and availability of alternative prey. The spiders were also free to immigrate throughout the season, buffering any potential by the beetles to reduce their numbers (Topping & Sunderland 1998).

It was predicted that there would be a positive relationship between predation risk and the proximity of the spiders' vertical spatial niche to the ground. This only held for relatively sedentary web-dependent species, with the beetles choosing *T. tenuis* over *B. gracilis*. *Erigone* spp. seemed to suffer predation in proportion to their abundance. *P. degeeri* also appeared to be preyed upon according to its abundance when adults and juveniles were considered, but less than expected when juveniles were excluded from the analysis. This suggests that predation of *P. degeeri* was mainly suffered by juveniles. Further work is needed to address the variation in spatial co-occurrence of spiders and their prey at the microhabitat scale. Predators and prey are known to be non-randomly distributed on the horizontal spatial plane (see section 4.1), even in relatively homogeneous fields, and this is likely to be a major factor governing patterns of predation (Bohan *et al.* 2000, Winder *et al.* 2001, 2005, Pearce & Zalucki 2006, Bell *et al.* 2010).

4. Field-scale reciprocal dynamics of crop quality, prey, predators and tillage regime in winter wheat

Abstract

Annual crops are populated by subterranean species or those that rapidly colonise fields. Snapshots into the dynamic processes therein can be gained by studying the relationships between environmental variables, crop yield and movements of mobile species. Understory microhabitat properties, crop yield, populations of linyphiid spiders and their herbivorous (aphid) and non-herbivorous (Collembola) prey were sampled in a field of winter wheat. The field was split between ECOtillage (MT) and conventional tillage (CT). The relationships between these variables and the different tillage regimes were analysed using a SADIE grid on three occasions during the growing season. Pairwise comparisons were made, also using SADIE, either between the spatial arrangement of different variables, or the same variable on different occasions. Overall, crop yield was positively related to aphid abundance. Despite its simpler microhabitat, the CT treatment yielded more wheat than the MT treatment and harboured higher numbers of individuals of most species, suggesting that populations were driven by resource, rather than microhabitat, availability. Spatial randomness of aphids and Collembola generally increased through the season. The distributions of all adult linyphiids were random, while the juveniles of *Tenuiphantes tenuis* and *Bathyphantes gracilis* began the season in clusters (CT treatment), before becoming random, then returning to clustered. Patches of abundant prey were predicted to result in local increases in predators, either through aggregation (which was predicted to manifest as local increases in adults) or a numerical response (manifested as local increases in juvenile populations). Juveniles of *B. gracilis* and *T. tenuis* increased in response to both aphids and collembola early in the season. Conversely, prey declines were expected in patches where predators were abundant. This was never observed for collembola, but early season aphid population decline was related to the clustering of juveniles of *B. gracilis*, *T. tenuiphantes* and *Erigone* spp. These lagged responses to prey concentrations suggest aphids and linyphiids are dynamically linked.

4.1. Introduction

4.1.1. Community assembly in arable crops

The apparent uniformity of an arable crop often belies a heterogeneous ecosystem, patches of which may differ markedly. Such differences may be abiotic (e.g. soil properties, moisture, light or temperature) or biotic (suitable prey, hosts or the absence of predators or parasites). These different elements will interact; species interactions will be determined by the environment (which itself consists, in part, of other species) and the environment reciprocally determined by the results of these species interactions (Vandermeer 1972). The degree to which patterning of spatial heterogeneity is observed inevitably depends on the scale at which it is sampled (Bohan *et al.* 2000, Winder *et al.* 1999). Furthermore, at a given scale, the mechanisms underlying these patterns may themselves operate at different scales to those at which the patterns are observed; they may be imposed by larger-scale constraints, or emerge from the interactions of many smaller-scale units (Levin 1992, Hassell *et al.* 1991). As the scale of observation is reduced, biotic factors, especially those concerned with the spatial movements of individuals, gain importance, while the influence of abiotic factors are thought to concomitantly decrease (Soberón & Nakamura 2009, Hortal *et al.* 2010). The likelihood, for example, of finding a spatial pattern when sampling a given species at the macro scale (>10 km) depends more on the air temperature (which may covary with precipitation), while at the micro scale (<10 m), it would depend more on the presence of a suitable prey species within its foraging range.

From an evolutionary perspective, the cultivation of arable crops began very recently, approximately 11,000 years ago (Zohary & Hopf 1993). In annual systems, the environment undergoes catastrophic change each year at harvest; but at a similar time each year (Wissinger 1997). Thus the assembly of the invertebrate community at the field scale in such predictably ephemeral habitats, rather than being determined by long-term adaptive actions (Gause 1934, Cracraft 1988, Legendre & Legendre 1998), is a stochastic process dependent on immigration and priority effects (Tilman 2004, Hubbell 2005), whereby ecological benefits are gained from an immigrants early arrival at a site. Such models of community assembly emphasise that local diversity is limited not by competitive interactions

among established species, but by competition between new immigrants and already-established species (Tilman 2004).

The dominant species in arable crops are those that either can exploit a subterranean refuge from annual habitat destruction, are highly dispersive, or reproduce quickly (or indeed possess all of these characteristics) (Alderweireldt 1994b, Thorbek *et al.* 2003). Wissinger (1997) argues that many of the species (particularly insects) that populate such systems are therefore characterised by pre-existing adaptations to such predictably ephemeral habitats (PEHs). Such species cyclically colonise refuge habitat and the ephemeral crop; they have generations that express K-selected traits in the former (seeking refuge and delaying reproduction) and r-selected traits in the latter (high fecundity, rapid development and early reproduction) (Wissinger 1997). Non-crop refugia for natural enemy species in arable crops include hedgerows (Thomas *et al.* 2001, Bell *et al.* 2002, Holland *et al.* 2009) and soil (Thomas *et al.* 2008), to fallow fields (Robinson & Sutherland 2002, Schoenly *et al.* 2010) and woodland (Kromp *et al.* 1999).

4.1.2. Spiders as natural enemies in annual crops

Linyphiid spiders are pre-adapted to PEHs in a number of ways. They are generalist predators, consuming a range of prey including aphids, collembola, diptera, thrips (Imhalsy & Nentwig 1995). They are all highly mobile and disperse using ballooning throughout their lives. Ballooning is a strategy thought to have arisen between ~135-65m years ago following the emergence of large numbers of grazers which led to habitat fragmentation and large areas of grassland (Bell *et al.* 2005). Ballooning involves climbing a plant and extruding a length of silk thread which then carries the spider to a new site some distance away by means of wind (Thomas *et al.* 2003). Varying dispersal abilities mean that a spider species' distributions in a crop depends largely on the proximity to source habitat of the crop and thus the environmental heterogeneity at a regional (Nyffeler & Sunderland 2003), landscape (Bianchi *et al.* 2006, Schmidt *et al.* 2008,) and farm scale (Lemke & Poehling 2002, Schmidt *et al.* 2008, Macfadyen *et al.* 2009), all of which influence the numbers of beneficial natural enemies available to immigrate into the crop (Topping & Sunderland 1998, Sunderland & Samu 2000). In most cases, the numbers of a given species in arable fields depends on the existence of greater areas of non-crop habitat in the surroundings, which is the case for

Bathyphantes gracilis and *Erigone* spp. (see Schmidt *et al.* 2008). The abundance of *Tenuiphantes tenuis*, on the other hand, is positively associated with the amount of surrounding arable crop habitat (Schmidt *et al.* 2008), which partially explains its high dominance of the arachnofauna in crops (chapter 3).

While high rates of dispersal are a necessary characteristic for the colonisation of arable crops, phenological synchronisation with the timing of the disturbance is highly influential (Samu & Szinetár 2002). High rates of reproduction and development following colonisation also characterise such species, enabling rapid population growth, with most linyphiids displaying multivoltine reproduction (De Keer & Maelfait 1988, Alderweireldt & De Keer 1990, Thorbek *et al.* 2003).

4.1.3. Cereal aphids as crop pests

In phytophagous species, high reproductive rates increase their potential as crop pests, particularly when they are capable of parthenogenesis (Hoffmann *et al.* 2008). Aphids are phloem feeders, capable of reducing crop yield (Larsson 2005, Goggin 2007), quality (Basky & Fonagy 2003) and acting as vectors of disease (Fieberg *et al.* 2004). Three species are particularly common in the cereal crops of Western and central Europe; the bird cherry oat aphid *Rhapalosiphum padi* and *Metapolophium dirhodum* overwinter on cherry trees and roses, respectively (Vickerman & Wratten 1989). *Sitobion avenae*, meanwhile, is monoecious to Gramineae, overwintering on wild grasses and/or autumn-sown crops (Vickerman & Wratten 1979, Leslie *et al.* 2009). The niches in the wheat tiller also differ between species. *S. avenae* prefer the upper leaves, moving to the head once the ears have emerged (Wratten 1975). A minority of *R. padi* feed on the head, but are mostly found in the lower parts including the sheath, of the upper leaves. *M. dirhodum*, meanwhile, usually feeds on the leaves, starting at the lower end of the plant and moving upward as the lower leaves senesce (Dean 1974).

Neither *R. padi*, *M. dirhodum* nor *S. avenae* are strictly asexual, rather each employs a strategy of cyclical parthenogenesis (Fig. 4.1) (Vickerman & Wratten 1979). They achieve the highest growth rates during the summer by mostly producing apterous offspring asexually in the crop, a reproductive mode which ensures that as much energy as possible is expended in reproduction (Newton & Dixon 1990). Reductions in plant quality and local crowding interact to produce

signals to give birth to winged morphs (alates), allowing the colonisation of new habitat (Dixon 1998). The onset of sexual reproduction is triggered by shortening day lengths (Simon *et al.* 1991). Eggs are more resilient to colder winters than virginoparae (sexual wingless females) (Simon *et al.* 2010), so sexually reproducing lineages are favoured in areas with regular harsh winters (Dedryver *et al.* 2008).

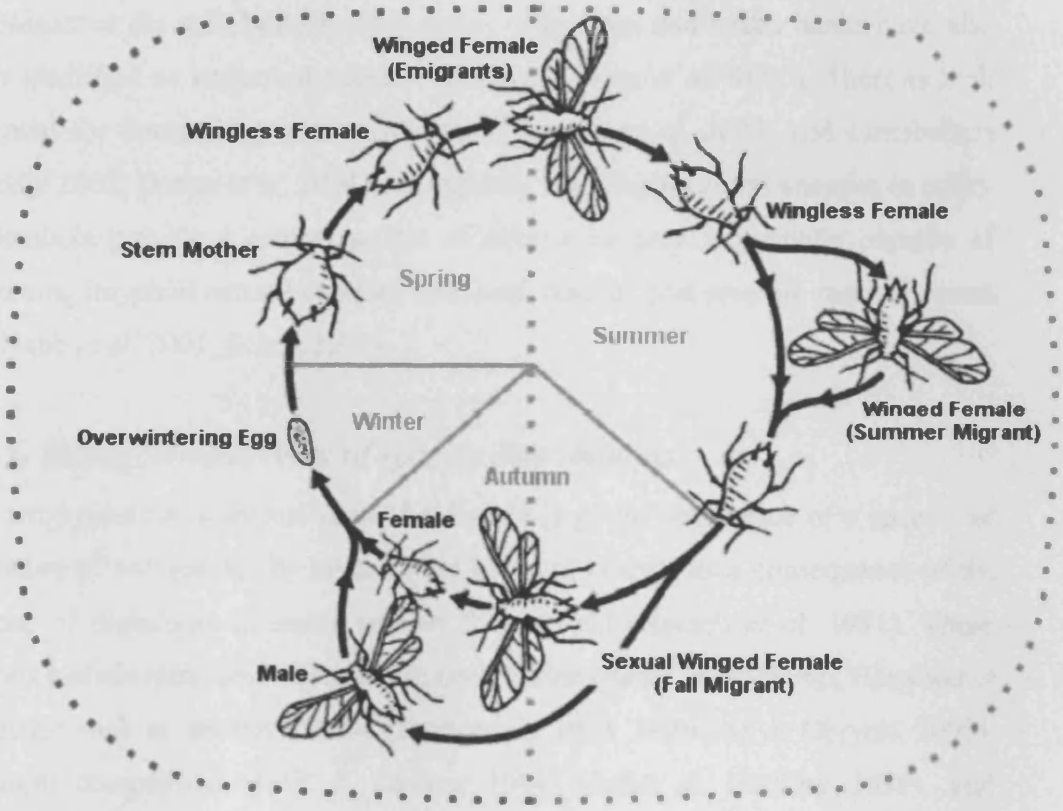


Fig. 4.1. A generalised lifecycle of a species with cyclical parthenogenesis. Green section shows the asexual phase, the red section the sexual phase. Canadian Ministry of Food and Rural Affairs (http://www.omafra.gov.on.ca/english/crops/facts/04-059.htm)

4.1.4. Collembola as alternative prey

The epigeic collembola of arable crops form a key link between the soil and above-ground food webs (Eisenhaur *et al.* 2010). As a group they are polyphagous consuming detritus, fungus and soil nematodes (Read *et al.* 2006). Populations of collembola on arable crops are also often numerically dominated by a small number of species, including for example *Lepidocyrtus* spp., *Isotoma* spp. and *Entomobrya multifasciata* (Alvarez *et al.* 2001, Agustì *et al.* 2003). Collembola can overwinter in the soil, but disturbed crops, hedgerows and beetle banks have also been identified as important source habitats (Alvarez *et al.* 2000). There is high potential for intraguild predation (chapter 3, Lucas *et al.* 2005) and cannibalism (Buddle 2002, Denno *et al.* 2004) among early colonizing natural enemies in crops. Collembola provide a critical source of alternative prey, potentially capable of sustaining linyphiid natural enemies until such time as pest prey are more apparent (McNabb *et al.* 2001, Scheu 2001).

4.1.5. Endogenous drivers of species distributions

Observed patterns of events at the field scale (e.g. the occurrence of a species or predation of one species by another) are likely to emerge as a consequence of the actions of organisms at scales smaller than a field (Hassell *et al.* 1991). These actions include intra- and interspecific competition (Samu *et al.* 1996b, Harwood *et al.* 2003) such as priority effects (Herberstein 1998, Harwood & Obrycki 2005), apparent competition (Holt & Lawton 1994, Muller & Godfray 1999), and predation (Schmidt *et al.* 2003, Holland *et al.* 2008, Harwood *et al.* 2004, 2005). The latter may include intraguild predation (Polis *et al.* 1989, Holt & Polis 1997) and cannibalism (McNabb *et al.* 2001, Wise *et al.* 2006). Species abundances of both herbivores and, indirectly, predators, may also depend on the nutritional value of a crop, which in turn relies on the crop's ability to fix these nutrients (Eisenhaur *et al.* 2010). Non-organic nitrogen sources notwithstanding, nutrient levels and uptake are governed by the abundance and diversity of subterranean decomposers, mycorrhizal fungi and nitrogen-fixing bacteria (van der Heijden *et al.* 1998, Lupwayi *et al.* 2004, Ke & Scheu 2008).

4.1.6. Exogenous drivers of species interactions

Those variables to do with soil biota are directly influenced by the tillage regime employed on a field. Conservation tillage regimes (also known as ECOtillage and minimum tillage) share the aims of improving soil structure and reducing manpower, energy input and soil erosion (Rieger *et al.* 2008) and disturbance (Thorbek & Bilde 2004). If these aims are successfully met, these changes usually also have the beneficial effect of stabilizing soil communities (Glover *et al.* 2010). Conservation tillage increases the abundance of collembola (Brennan *et al.* 2006) and spiders (Holland & Reynolds 2003), but predicting whether this may increase crop yield is difficult because more effective herbivore suppression as a result of increased natural enemy abundance may be counteracted by the predators consumption of these non-pest prey. Rieger *et al.* (2008) found that a minimum tillage regime, whilst benefitting the farmer finanically by lowering economic inputs in terms of fuel and labour, produced yields equal to a conventionally managed field, especially when wheat was preceded by oil seed rape as opposed to maize. Verch *et al.* (2008) compared the economic costs of different regimes, and found that over a four-year rotation including winter wheat, a minimum tillage regime was more profitable than conventional ploughing.

Other important anthropogenic inputs include the application of pesticides (Chiverton & Sotherton 1991, Frampton & Dorne 2001, Bell *et al.* 2002), fertilisers (Stoate *et al.* 2001, Muller *et al.* 2005) and detrital subsidies (Halaj & Wise 2002, Bell *et al.* 2008, von Berg *et al.* 2010). However, the stated aim of many government initiatives, e.g. the Common Agricultural Policy in Europe and Glastîr in Wales, is to reduce chemical inputs while maintaining crop yield, in order to increase the sustainability of food production (section 1.2).

4.1.7. Analytical tools

By taking 'snapshots' of food webs or spatial proximities at different times during a season, temporal changes in trophic interactions may be established or inferred (Bell *et al.* 2010). If individuals of a prey species form aggregation(s) (section 4.3.3.) in a particular part or parts of a field, a predator species may subsequently display a positive growth rate in this locality. This suggests a local aggregative response by the predators to the prey clusters and has been demonstrated experimentally (Winder *et al.* 2001, 2005). Similarly, a negative effect of predation

on prey populations is indicated when local clustering of a predator species within a field coincides with a local decline in a prey species.

While the processes that dominate interactions at field scales are uncertain (Winder *et al.* 1999, Bohan *et al.* 2000), it would be expected that the heterogeneity of local processes such as predation are key given the relative homogeneity of the biota as a whole (i.e. the crop itself) in arable fields (Bell *et al.* 2010). Indeed, molecular studies of predator gut contents have, by comparing the incidence of predation with prey responses, provided evidence that predation is a driver of spatial patterns. Winder *et al.* (2005) found phased patterns of co-occurrence between the carabid predators *Pterostichus melanarius* and *P. madidus* and their aphid prey *S. avenae* using SADIE analysis. Gut content screening with ELISA (see Symondson 2002) suggested predation drove this phasing, because a positive relationship was found between the likelihood of finding prey remains and their activity-density in the proximity of their prey. Bohan *et al.* (2000) showed a similar pattern between the carabid predator *P. melanarius* and the slugs *Deroceras reticulatum* and *Arion* spp. Similarly, the spatial co-occurrence of *Pterostichus* spp. beetles and earthworms was positively related to the probablility of finding a given species of earthworm's DNA in the beetles' guts, as measured by PCR (Bell *et al.* 2010).

4.2. Aims and objectives

The effects of variation at the producer level (crop quality; bottom-up effects) were expected to ramify through the food web, causing reciprocal interactions between this crop quality and the dynamics of a number of critical components of the food webs' mobile biotic elements (aphids, collembola and predatory linyphiid spiders). These interactions were analysed in the context of a split plot experimental design, where each half of the experimental area was subjected to a different tillage regime (Hatten *et al.* 2007, Holland & Reynolds 2003).The following null hypotheses were tested:

Effects of tillage regime: i) understorey microhabitat and crop yield are unaffected by differences in the tillage treatment, ii) where differences do occur, these will not ramify through the food web to affect herbivore abundance.

It was expected that the rapid growth rates and dispersal strategies of the species that were monitored would result in a contiually shifting mosaic of patch dynamics consistent with ephemeral habitats (Vandermeer 1972, Hassell *et al.* 1991). Thus, the null hypothesis was tested that the spatial arrangements of aphids, collembola and each linyphiid species are i) random and ii) persistent over the duration of the experiment.

Where aphids are exploiting a nutritious host, they produce mostly non-winged, asexual offspring. Linyphiid spiders are relatively more mobile than apterous aphids and their collembolan prey, so localities where such prey cluster may attract or retain mobile predators to forage (Harwood *et al.* 2003, Thomas *et al.* 2003, Bell *et al.* 2005). It was therefore expected that aggregation to such prey would be evident, followed by exploitation of this prey. If such exploitation occurred it was predicted to result in the subsequent decline of prey and concomitant rise in predators (Winder *et al.* 2001, 2005). A rise in juveniles would suggest a numerical response to the prey, while an increase in the number of adult predators would suggest an aggregative response. Thus, the null hypothesis that no significant relationship exists between the local cluster coefficients of prey taxa (aphids and collembola) and the subsequent change in predator population (e.g. June prey clustering v July-June predators) was tested.

Conversely, local prey abundance may drop in response to the clustering of mobile predators, either as a direct result of predation or through emigration. The null hypothesis of no relationship between local prey clustering and subsequent changes in prey populations at the corresponding sampling point was also tested.

4.3. Methods

4.3.1. Sample collection

Small arthropods (including spiders, aphids, collembola, diptera and small carabid and rove beetles) were sampled by Vortis sampler (Burkhard Ltd, Rickmansworth, UK) from: 10 sucks, each lasting 3 s, were taken covering an estimated sampling area of 0.18 m^2, which was followed by a hand-search of the top layer of soil. The 10 samples were then bulked and transferred to a plastic bag and placed inside a cool box on ice. In the laboratory, each sample was live-sorted from the detritus and stored in 100% ethanol. In addition to the aphids collected by Vortis sampler, aphids occupying the heads of 20 wheat tillers per sampling point were also included in the samples. At each point in the grid, samples were taken at three stages: flowering (week beginning 12 June: Zadoks scale 69-70); milky or mealy ripe ears (week beginning 10 July: Zadoks scale 73-85) and at harvest (week beginning 31 July: Zadoks scale 90-92). Adult spiders were identified to species by their morphology, while juveniles were identified using species-specific primers in multiplex PCR (section 3.2.3, Table 3.2, chapter 3).

4.3.2. Field site and tillage regimes

Sampling took place in Highfield (4.98 ha) at Rothamsted Research Institute, Harpenden, Herts, (Highfield 4.98 ha, lat: 51.803N, long: 0.364W), which has been farmed under a CT-MT till (the soil treatments are hereafter referred to as MT - minimum till and CT - conventional ploughing) regime since August 2004 (the remaining half of the field having been used for ley-arable treatments, i.e. grass meadow). The CT-MT is an asymmetric split-plot field comprising about 2.5 ha of experimental area in total (MT = 1.45 ha 'oblong-shaped'; CT = 1.1 ha 'rough triangle') over which a sampling grid of 80 points was superimposed (Fig. 4.2). In 2004, the field was planted with winter oilseed rape, which was rotated to winter wheat for the 2006 harvest. The MT half of the field was cultivated using the 'Cultipress' (Simba International, Sleaford, UK) which cultivates, levels and consolidates the seedbed (Fig. 4.3), to a depth of 3-5 cm. This was passed twice, followed by direct drilling of the winter wheat cultivar Consort™ at a rate of 300.00seeds/m^2, pre-treated with Redigo Twin™ a fungicide, and then rolled. Additionally, the CT area was ploughed using a conventional six furrow plough to

a depth of ~25 cm before the use of the cultipress then power harrowed with an Accord combination drill to level and drill the seedbed. The type and rate of seed drilling was the same as the minimum tillage area.

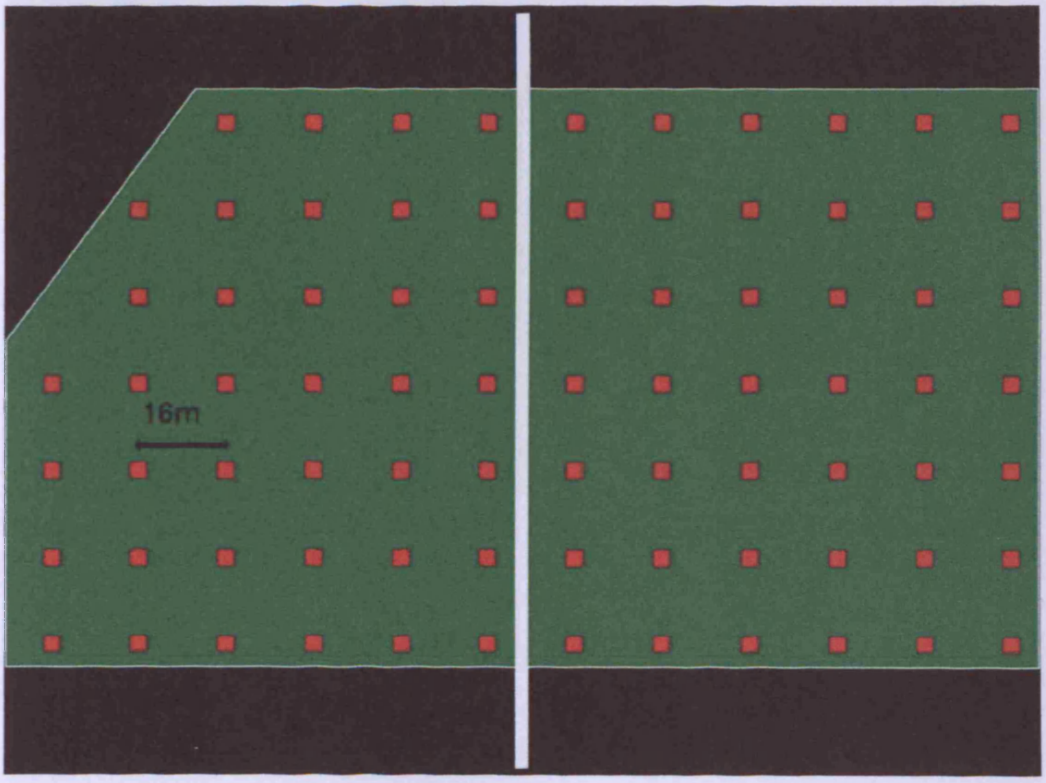


Fig. 4.2. Map showing the layout of the sampling grid. Each red dot represents a sampling site. The green area on the left hand side of the dividing line is the minimum tillage (MT) area, the right hand side the conventional tillage (CT).

Fig. 4.3. Soil for the minimum tillage treatment was prepared using the 3.3 m Simba Cultipress (pictured).

4.3.3. SADIE

Spatial Analysis by Distance IndicEs (SADIE) (Perry 1995) is a method of assessing the magnitude of non-randomness of spatial distributions. It is a technique explicitly designed for dealing with spatially referenced counts of events, such as individuals or ratios that accommodates counts of zero (Perry 1998). The indices are predicated on algorithms which calculate the minimum effort, in terms of distance travelled, of moving the actual data to hypothetical extremes of distributions. Thus, the distance to regularity (D) measures the minimum amount of movement a population would need to make for all quadrat counts to be equal[8]. Thus, small values of D (and large values of C, see below) would suggest spatially regular patterns. The degree of regularity may be formally tested using random permutations of the movement of units, the arithmetic mean of which is denoted E_a. An index of aggregation, I_a, is derived from D/E_a. In cases where I_a equals 1, counts are randomly arranged on the grid, while if $I_a > 1$ counts are aggregated into clusters, and when $I_a < 1$ counts conform to a regular arrangement. The measure P_a

[8] Distance to crowding (C) is the converse of D (Perry 1995); it represents the effort required for the individuals in a population to congregate in the spatial unit with the lowest value. However, this measure is only reliable if there exists only one cluster (either a patch or gap) in the grid.

represents the proportion of randomly permuted values of distance to regularity that is larger than the observed value, D. A two-tailed test of significance can be applied to test whether the spatial occurrence of events is spatially regular. Where the significance level, α, is set at 0.05, the null hypothesis of randomness can therefore be rejected when $P_a < 0.025$ (aggregation) or > 0.975 (regularity or disaggregation).

Clusters may take the form of either patches or gaps. Patches are neighbourhoods of units where the counts are larger than the overall grid mean and gaps are where it is lower. Where a unit forms part of a patch (i.e. has a count, c_i, greater than the mean, m) it is assigned a patch index, v_i. Gaps are neighbourhoods where counts are lower than the grid mean ($c_i<m$) and assigned a gap index (v_j). Values of v_i and v_j of +1.0 and -1.0, respectively, conform to a random arrangement of the counts. Clusters were mapped by interpolating contours around values of cluster indices using the Kriging method (Webster & Oliver 1989) in Surfer v9.0 (Golden Software Ic, Golden, Colorado, US). Patches (where $v_i > 1.5$) were shaded red, while gaps (where $v_j < -1.5$) were shaded blue (Perry *et al.* 1999). Hence, the coloured parts of the red-blue plots represent localities where clustering is half as great as that expected by chance.

Two populations of events may be spatially associated, disassociated or occur at random with respect to each other (Perry 1998). Formal testing of this was based on a measure of local spatial association, denoted by the index χ_k, which represents the similarity between the clustering index of the two populations at the kth unit (Winder *et al.* 2001):

$$\chi_k = n(z_{k1} - q_1)(z_{k2} - q_2) / [\sum_k (z_{k1} - q_1)^2 \sum_k (z_{k2} - q_2)^2]^{1/2} \qquad \text{eq.1.1}$$

where the n indices of the first set are denoted z_{k1} with a mean of q_1, while the second are denoted by z_{k2} with a mean of q_2 (Winder *et al.* 2001). Positive values arise from coincidence of positive or negative units, negative values from coincidences of opposite cluster types. Overall spatial association, the test statistic SADIE X, was calculated from the mean of the local values

$$X = \sum_k \chi_k / n \qquad \text{eq. 1.2}$$

Significance of X was tested by randomly reassigning values of z_k among the units after allowing for small-scale autocorrelation by the method of Dutilleul (1993), which reduces the effective sample size and therefore degrees of freedom for the analysis (i.e. n in eq. 1.2). For each comparison, a random seed number was generated, and the maximum 999 permutations were run (Perry *et al.* 1999).

Standard measures of spatial heterogeneity such as correlograms (see Perry 2002) or Taylor's power law (Taylor 1984, Kendal 2004), simply measure skew in the distribution of counts at points or quadrats i.e. the statistical heterogeneity of a number of samples. SADIE retains the spatial information, producing a measure of spatial non-randomness from the aggregation of counts into clusters, and the regularity of those counts. Additionally, SADIE deliberately downweighs the effects of isolated extreme values. It provides a combination of a formal, overall randomisation test of spatial non-randomness using the indices I_a , v_i and v_j and a more intuitive spatial map of clustering using red-blue plots (Perry *et al.* 1999). When spatial co-occurrence, either between two sets of events at the same time, or the same events at different times is tested, this may be similarly mapped using plum-green plots (Winder *et al.* 2001) and tested formally with an adjustment for autocorrelation (Dutilleil 1993).

In all cases, SADIEShell v1.22 was used for SADIE analyses. The parametric rbrelv13.exe was used for cluster analyses, while association analyses were carried out with the 'Quick Association' programme n_a.exe (Winder *et al.* 2005). Regression analyses and pairwise comparison tests were all carried out using R version 2.10.1 (R Core Development Team 2010). Interactions were only fitted if they were thought to be informative and biologically meaningful to help reduce the problem of multiplicity of P-values (Grafen & Hails 2002). Simplification of the regression model proceeded via stepwise deletion of non-significant terms (Crawley 2007), with comparison of the P-value of the likelihood ratio statistic and the Akaike Information Criterion (AIC) at each step (Pinheiro & Bates 2000).

4.3.4. Relationship between environmental variables and crop yield

Levels of crop yield were estimated just before harvest; 10 ears per sample point were removed, dried and weighed, before being transposed into yield/m^2/point through multiplication by the number of tillers per 0.5 m^2. SADIE analysis requires intergerised counts, so the crop yield in g/m^2 was analysed. The proportions of algae, bare ground and organic matter were surveyed at each sample point (a square metre of ground was surveyed and each cover type estimated to the nearest 5%). Soil moisture is generally regarded as inversely proportional to its hardness. This latter was estimated by the use of a penetrometer (Findlay Irvine Ltd, Midlothian) fitted with a small cone (12.8 mm diameter). At each sampling point, five separate soil profiles were measured. For each replicate, a reading of the force (in Kgf) necessary to penetrate the soil was taken at every 1.5 cm to a depth of 7.5 cm. The mean readings from each sampling point were compressed, using principal components analysis (GenStat, VSN International), into a single value for each horizon.

Parametric SADIE cluster analysis was performed for each variable, and co-occurrence of each environmental variable was compared to the yield. Additionally, regression analyses were carried out to assess the relationship between cluster coefficients of each environmental variable and crop yield.

4.3.5. Comparisons of crop yield and invertebrate populations between tillage regimes

In cases where data conformed to the relevant assumptions, t-tests were carried out to compare the mean populations of invertebrates and levels of crop yield per sample point between the two tillage treatments (most values required a square root transformation before these tests could be undertaken). For data that could not be normalised following transformation, Wilcoxon Ranks Sum Tests were performed (Crawley 2007). A stepwise regression (see section 2.6) was carried out to evaluate the effects of both classes of herbivores (Orange Blossom Wheat Midge *Sitodiplosis mosellana* and aphids) on crop yield.

4.3.6. Predator and prey spatial aggregation and temporal flux

Parametric SADIE cluster analysis was undertaken for aphids, collembola and total populations of each spider and a red-blue plot was constructed for each. Temporal stability of species was tested by comparing clustering coefficients of each species at every sampling period (i.e. June-July or July-August) with those of the subsequent sampling period.

4.3.7. Prey response to predator clustering

An explicit spatial analysis of the response of aphids and epigeic collembola to the presence of spiders was carried out. Local cluster coefficients (v_i or v_j) of predators were calculated from SADIE analyses of their counts. Logarithmic growth rates (r_p) of prey (aphids and collembola) were calculated using the formula:

$$r_p = \ln(n_{kpt+1}) - \ln(n_{kpt}) \qquad \text{eq.1.3.}$$

where n represents the count, at point k, of species p, on date t. These were regressed against local SADIE cluster coefficients, which, being independent of predator density, relates prey growth to true predator clustering. Separate analyses were undertaken to assess the responses of i) adult spiders plus juveniles ii) adult spiders alone and iii) juveniles including subadults, of each spider species.

4.3.8. Numerical responses of predators to prey

The numerical responses of spiders to aggregations of prey were estimated by regressing subsequent growth rates of predators against SADIE cluster coefficients (v_i or v_j) of aphids and collembola. Growth rates of predators (r_{PR}) were calculated as:

$$r_{PR} = N_{kPRt+1} - N_{kPRt} \qquad \text{eq. 1.4.}$$

where N represents the count (at point k), of predator species PR, on date t. The approach to these regression analyses differs from that of Winder *et al.* (2001) in that patch (v_i) and gap (v_j) cluster coefficients are regressed in a single slope. This seems appropriate given that the indices represent points along a single interval scale (Legendre & Legendre 1998). Again, separate analyses were undertaken for

sampled counts of i) adults plus juveniles ii) adults alone and iii) juveniles including subadults, of each predator species.

4.4. Results

4.4.1. Crop yield and environmental measurements

Overall, MT (minimum tillage, conservation tillage) was significantly associated with lower crop yield, higher organic matter at the soil surface and lower soil moisture. Conversely, CT (conventional tillage, ploughing) was associated with higher crop yield, low surface organic matter, and soft, moist soil.

Levels of wheat yield show significant spatial patterning (Fig. 4.4). Highly significant spatial clustering corresponds to the tillage treatments (I_a = 1.892, P_a = 0.0012). A highly significant gap (v_j = -1.862, $P_{vj} < 0.001$) is present in the MT region, while a highly significant patch defines the conventionally tilled region (v_i = 1.965, $P_{vi} < 0.001$).

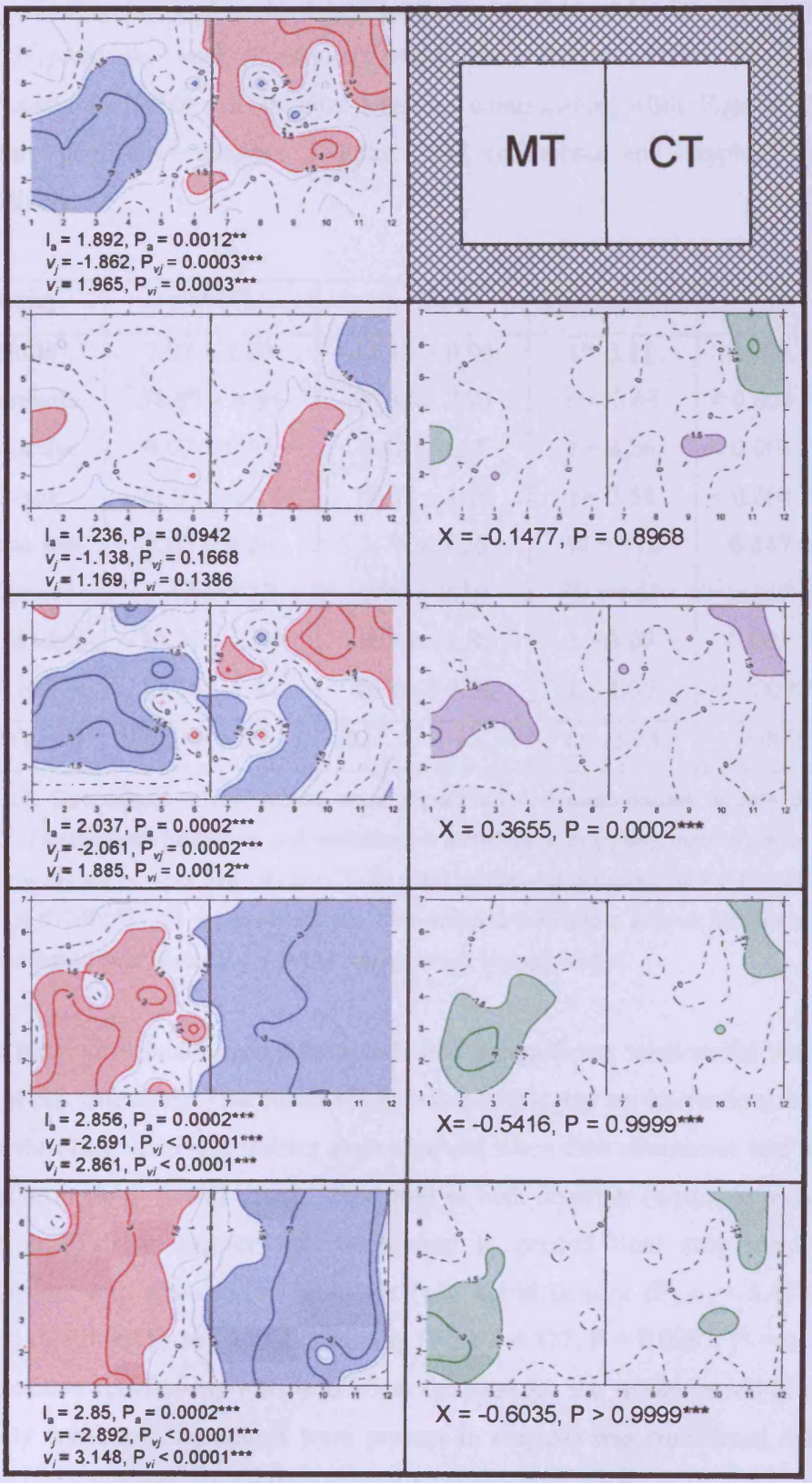
Under CT, understorey microhabitat broadly consisted of a mixture of algae and bare ground, whereas under MT, organic matter, which was mainly composed of rape stalks, seemed to dominate (Fig. 4.4). The analysis indicated no spatial clustering in the proportion of algae (I_a = 1.236, P_a = 0.0942) and no significant spatial overlap with algae and crop yield (X = 0.1477, P = 0.8968). Strong, inversely related, patterns were apparent in the proportions of bare ground and organic matter. Both showed significant clustering, with the most marked relationship existing between the tillage regimes and the proportion of organic cover, where the part of the field subjected to CT was entirely clustered into a single gap (v_j = -2.691, $P_{vj} < 0.001$). The proportion of bare ground at each sampling point showed an overall significant association with crop yield (X = 0.3655, P = 0.0002), while the proportion of organic matter covering the ground was significantly disassociated from crop yield (X = -0.5416, P = 0.9999). Soil hardness was similarly split according to the tillage regime and also disassociated from crop yield (X = -0.6035, P > 0.9999).

Additionally, a spatially explicit regression analysis, comparing crop yield and the SADIE cluster coefficients of percentage ground cover (Fig. 4.4) at each sampling point, also indicated a negative relationship between yield and the proportion of

organic material present ($F_{1,77}$ = 16.36, P < 0.001, r^2 = 0.17), while yield was unrelated to the proportion of algae ($F_{1,77}$ = 0.30, P = 0.585, r^2 = 0.003) and bare ground ($F_{1,77}$ = 1.78, P = 0.186, r^2 = 0.02).

Fig. 4.4 (overleaf). Yield v environmental measure.The left hand column shows SADIE red-blue plots for the wheat yield and environmental parameters across the whole experimental area. Each point on the grid is 16 m distant, therefore each sampling point effectively represents the centre of a square 16m x 16m. Contours are interpolated using Kriging (Webster & Oliver 1989). Clusters are defined as areas enclosed by contour levels of +1.5 (patch, red shading) or -1.5 (gap, blue shading). These indicate clustering (v_i > 1.5 or v_j < 1.5) half as great again as that expected by chance. Red-blue plots are accompanied by the overall clustering index (I_a), gap index (v_j) and patch index (v_i) along with probabilities (P_a, P_{vj}, P_{vi}, respectively) that these levels of clustering fall within the tails of the distributions of random permutations of the data (two-tailed test: *α = 0.05 P < 0.025 or P > 0.975; **α = 0.01; P < 0.005 or P > 0.995; ***α = 0.001, P < 0.0005 or P > 0.9995). The right hand column contains plum-green plots showing associations (plum areas) and disassociations (green areas) between crop yield and each of the microhabitat parameters. SADIE X is an overall index of co-occurrence; positive values suggest co-occurrence of aggregations, negative values represent dissociated patches, while values close to zero indicate a random arrangement. P denotes the probablility that X falls within the tails of the distributions of 999 random permutations of the cluster coefficient pairings (2-tailed test, as above).

Wheat yield (dry weight, g/m²)



4.4.2. Crop yield and invertebrate populations

Over the season, the mean abundances of invertebrates (both predators and prey) under CT were, in most cases, significantly higher than under the MT treatment. Only *Erigone* spp. and *P. degeeri* were less numerous under CT, but not significantly so. Table 4.1 contains details of comparisons, while Figures 4.5 and 4.6 show population changes in aphids and collembola and linyphiid spiders, respectively.

Species	n (MT)	n (CT)	Test statistic	P
Aphids	7.97 ± 1.03	11.45 ± 0.96	t = 3.11	0.003 **
Collembola	58.89 ± 6.85	96.36 ± 7.71	t = -3.63	< 0.001 ***
B. gracilis	4.02 ± 0.51	6.95 ± 0.55	t = 4.26	< 0.001 ***
T.tenuis	11.95 ± 1.14	18.02 ± 1.29	t = 3.58	< 0.001 ***
Erigone spp.	2.05 ± 0.36	1.76 ± 0.26	W = 778	0.847 ns
P. degeeri	1.47 ± 0.27	0.90 ± 0.16	W = 651	0.137 ns
Total spiders	30.21 ± 2.00	38.76 ± 1.80	t = 3.09	0.003 **
OWBM	13.95 ± 1.71	29.10 ± 3.58	t = 4.05	< 0.001 ***
Yield (g/m^2)	1050.83 ± 32.53	1231.50 ± 45.34	t = -3.24	= 0.002 **

Table 4.1. Comparisons of total annual mean populations (n = mean number of individuals per sample) of invertebrate herbivores and predators per sampling point (± SE) under minimum tillage (MT) (n = 38) and conventional tillage (CT) (n = 42) regimes. Significance values: P < 0.05*, P < 0.01**, P < 0.001***, ns = non-significant. Test statistics were either derived from t-tests (t) or Wilcoxon Ranks Sum Tests (W). OWBM: orange wheat blossom midge.

Of all herbivores examined, only aphids had a significant relationship with crop yield in any direction. This relationship was positive and no interactions between any of the other herbivore species were apparent when their abundance was used to predict crop yield. Aphid counts, expressed as both densities (n/sample point) and cluster coefficients (v_{ik} or v_{jk}), were used to predict final crop yield. This relationship was positive (in June) for both aphid density ($F_{1,77}$ = 5.484, P = 0.0218, r^2 = 0.0665) and aphid clustering ($F_{1,77}$ = 4.377, P = 0.0397, r^2 = 0.0538). This positive relationship also held when the total for the whole period (i.e. June and July combined, no aphids were present in August) was considered, whether density ($F_{1,77}$ = 4.672 , P = 0.034, r^2 = 0.0572) or aphid cluster coefficients ($F_{1,77}$ = 10.08, P = 0.0021, r^2 = 0.1158) (Fig.s 4.7, 4.8 and 4.9) were used as the explanatory

variable. Where July counts were used to predict yield, however, the relationship was non-significant for both aphid density ($F_{1,77} = 0.3278$, $P = 0.569$, $r^2 = 0.0042$) and aphid cluster coefficients ($F_{1,77} = 0.772$, $P = 0.382$, $r^2 = 0.1158$) despite higher abundances than June.

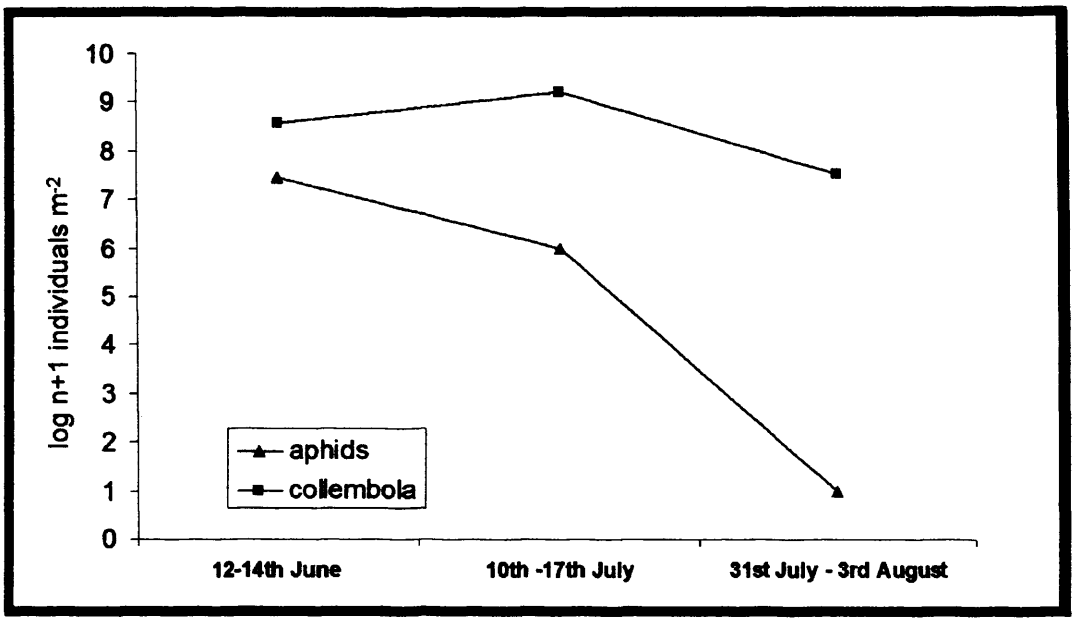


Fig. 4.5. Log-transformed (natural log) populations of isotomid collembola and aphids at each sampling date.

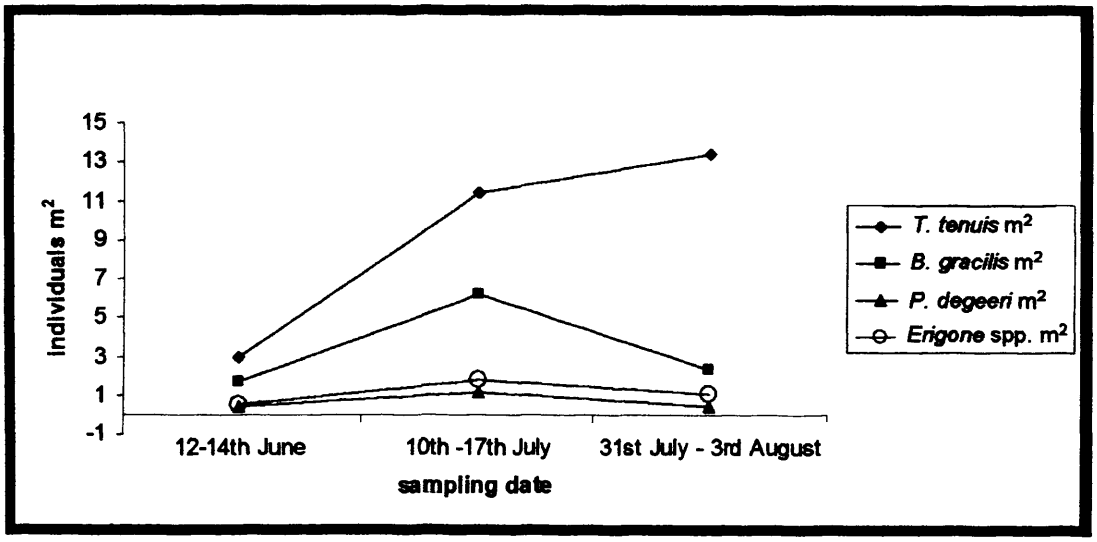


Fig. 4.6. Mean abundance (individuals m^{-2}) of the four commonest linyphiid spider species found in the wheat field at each sampling date.

Aphid populations were low during 2006, but still represented a greater proportion of biomass than collembola (Bell *et al.* 2010).

4.4.3. Spatial patterning and temporal flux of prey species

Red-blue plots for each species at each sampling occasion, and plum-green plots representing the co-occurrence of each species are shown in Figures 4.7, 4.8 and 4.9). Temporal co-occurrence maps for each species are shown in Fig. 4.10. Significant spatial clustering occurred in both aphids (I_a = 1.644, P_a = 0.007) and collembola (I_a = 2.446, P_a = 0.0002) during June, with each species broadly clustered in relation to the two tillage regimes. Both species displayed patches in the CT regime and gaps in the MT (Fig. 4.7). As the season progressed, however, their distributions showed progressively less clustering. Change in spatial distribution was pronounced in aphids, as indicated by a low co-occurrence index between June and July (X = -0.1899, P = 0.9536), which bordered on significance. By August no aphids could be found.

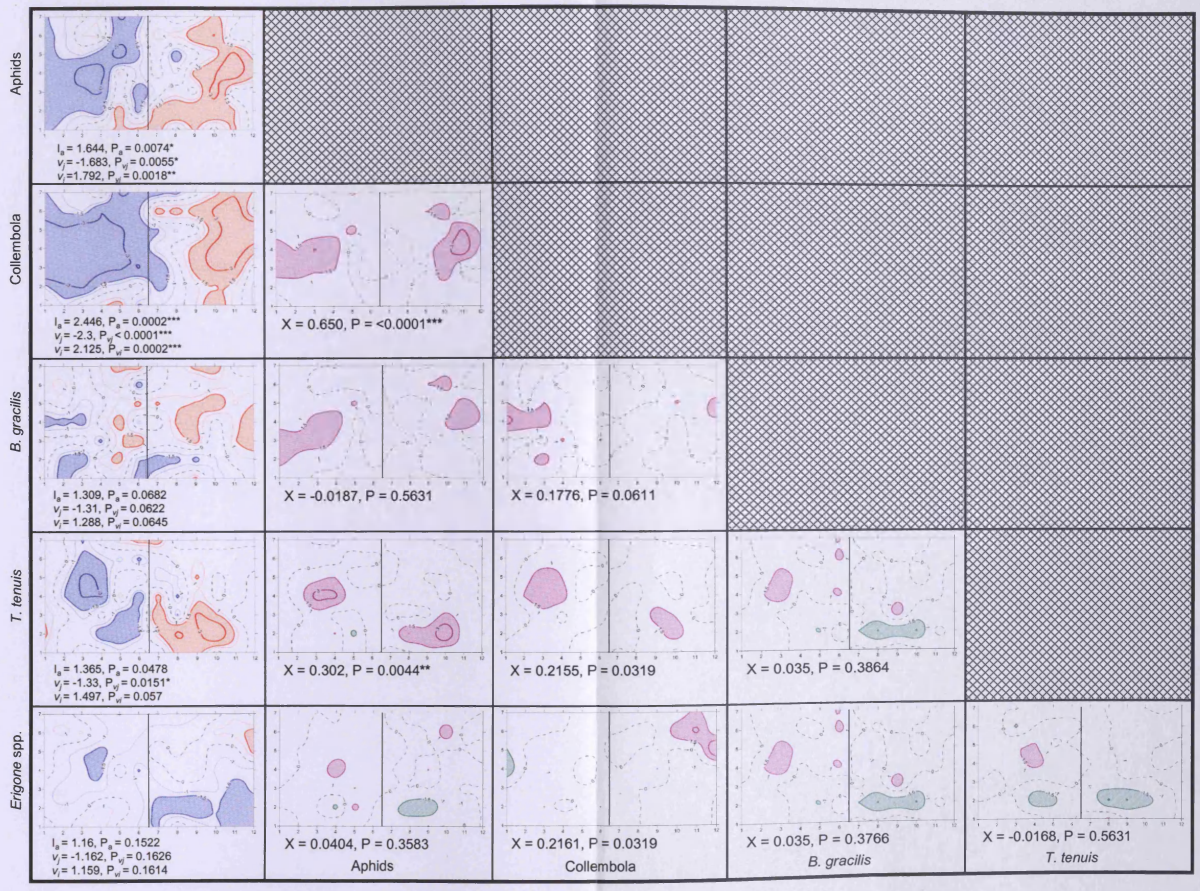
Across the year, spatial patterns of collembola changed from aggregated to random (Fig.s 4.7, 4.8, 4.9). In June they were highly significantly aggregated (I_a = 2.446, P_a < 0.001), a large gap corresponding with the MT treatment (v_j = -2.3, P_{vj} < 0.001) and a significant patch to the CT regime (v_i = 2.125, P_{vi} < 0.001). Overlap between June and July was high (X = 0.257, P = 0.0162), but no association existed between July and August's counts (X = -0.0599, P = 0.6619), with this later period witnessing a shift from borderline non-significant clustering in July (I_a = 1.458, P_a = 0.026) to a random distribution during August (I_a = 1.063, P_a = 0.2893).

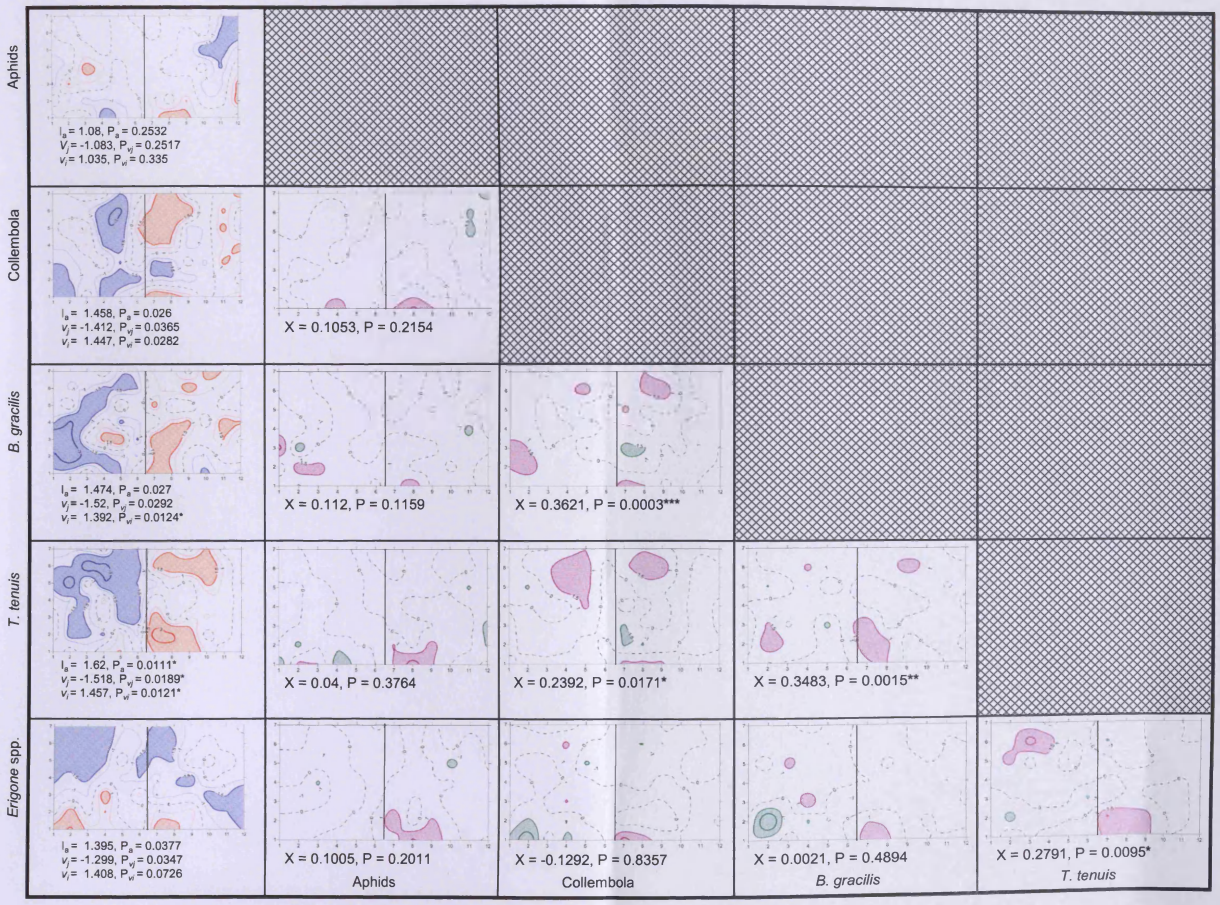
Fig. 4.7 (p87). June SADIE plots.The left hand side shows the SADIE red-blue plots for each trophic group, while the matrix contains the plum-green plots depicting the pairwise interactions of each species across the whole experimental area for flowering wheat heads (week beginning 12 June: Zadoks scale 69-70)

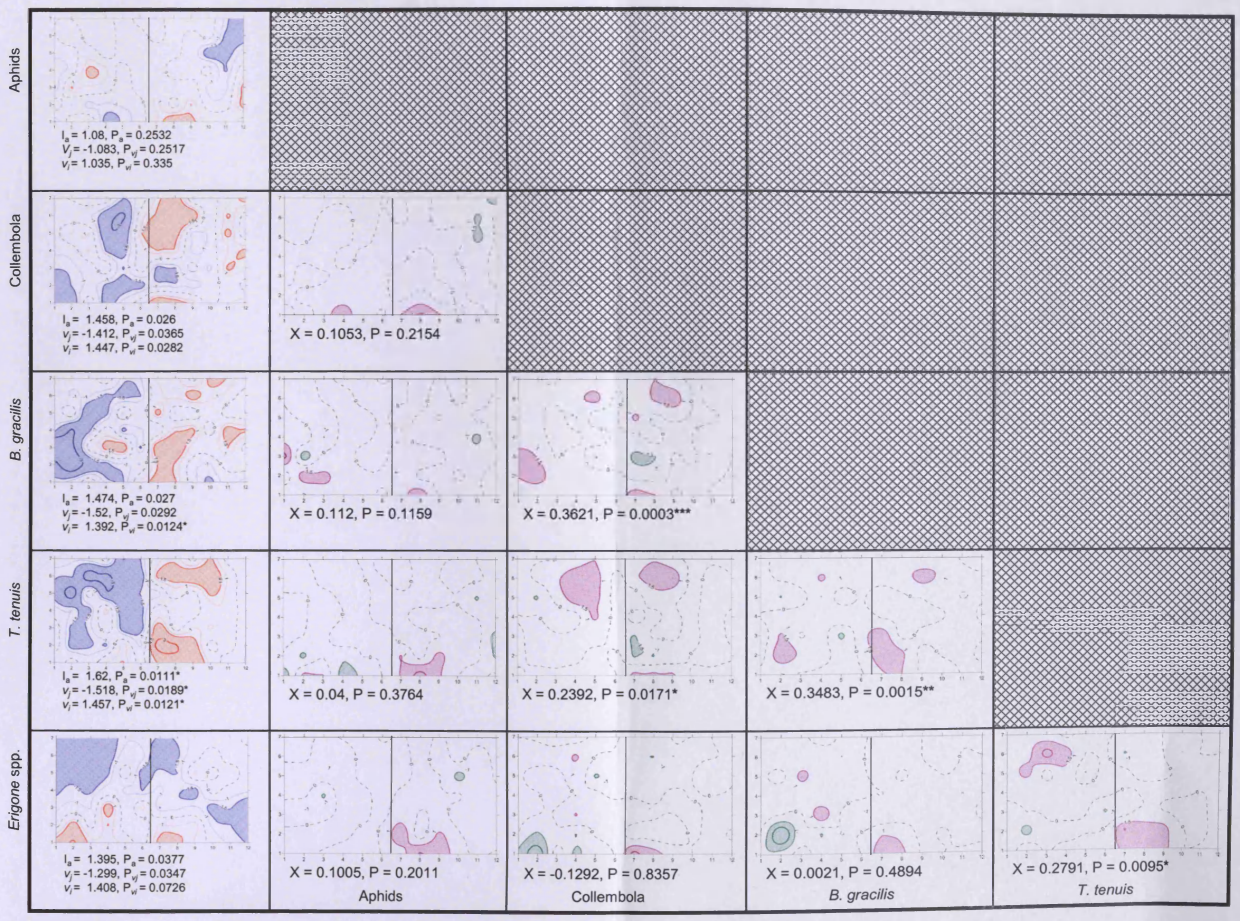
Fig. 4.8 (p88). July SADIE plots. The left hand side shows the SADIE red-blue plots for each trophic group, while the matrix contains the plum-green plots depicting the pairwise interactions of each species across the whole experimental area for milky or mealy ripe ears (week beginning 10 July: Zadoks scale 73-85).

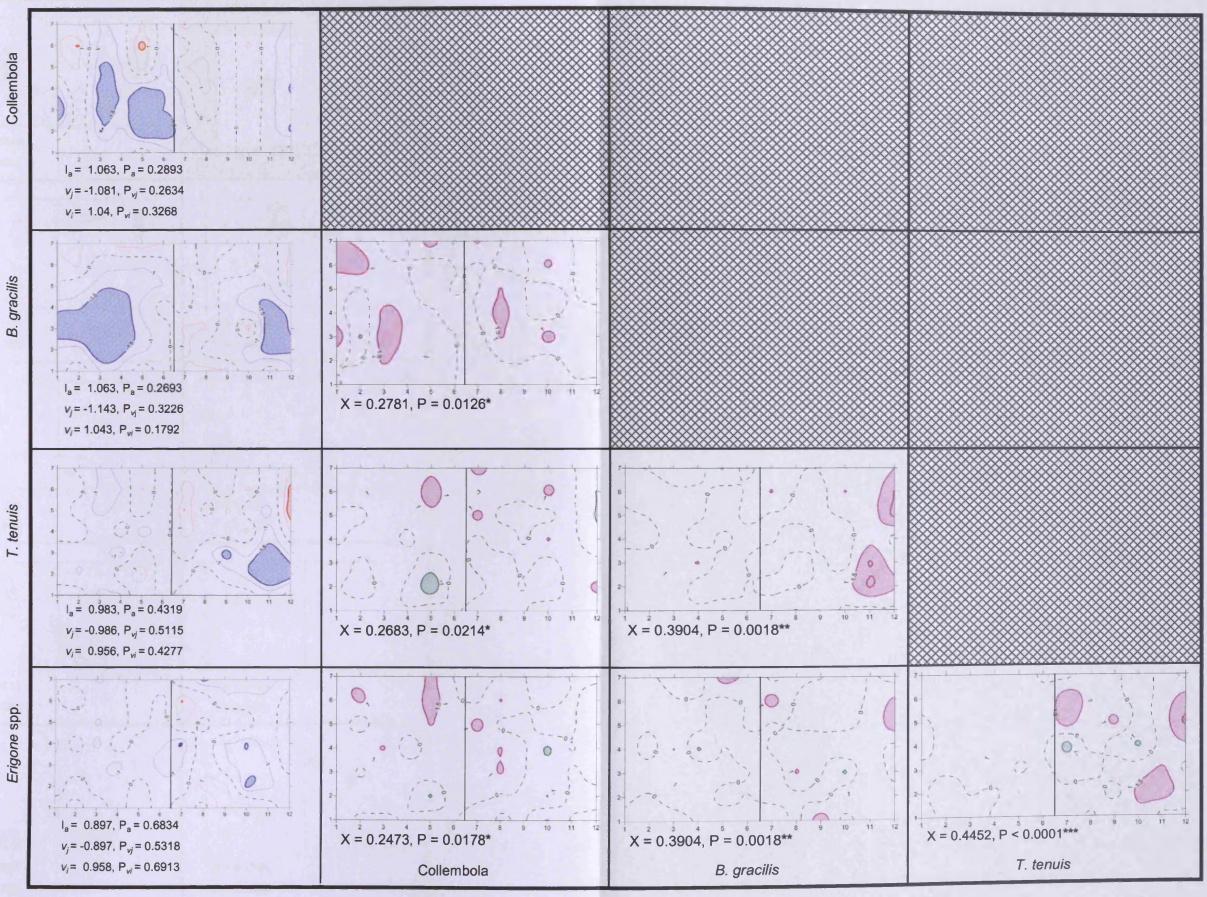
Fig. 4.9 (p89). August SADIE plots. The left hand side shows the SADIE red-blue plots for each trophic group, while the matrix contains the plum-green plots depicting the pairwise interactions of each species across the whole experimental area at harvest (week beginning 31 July: Zadoks scale 90-92).

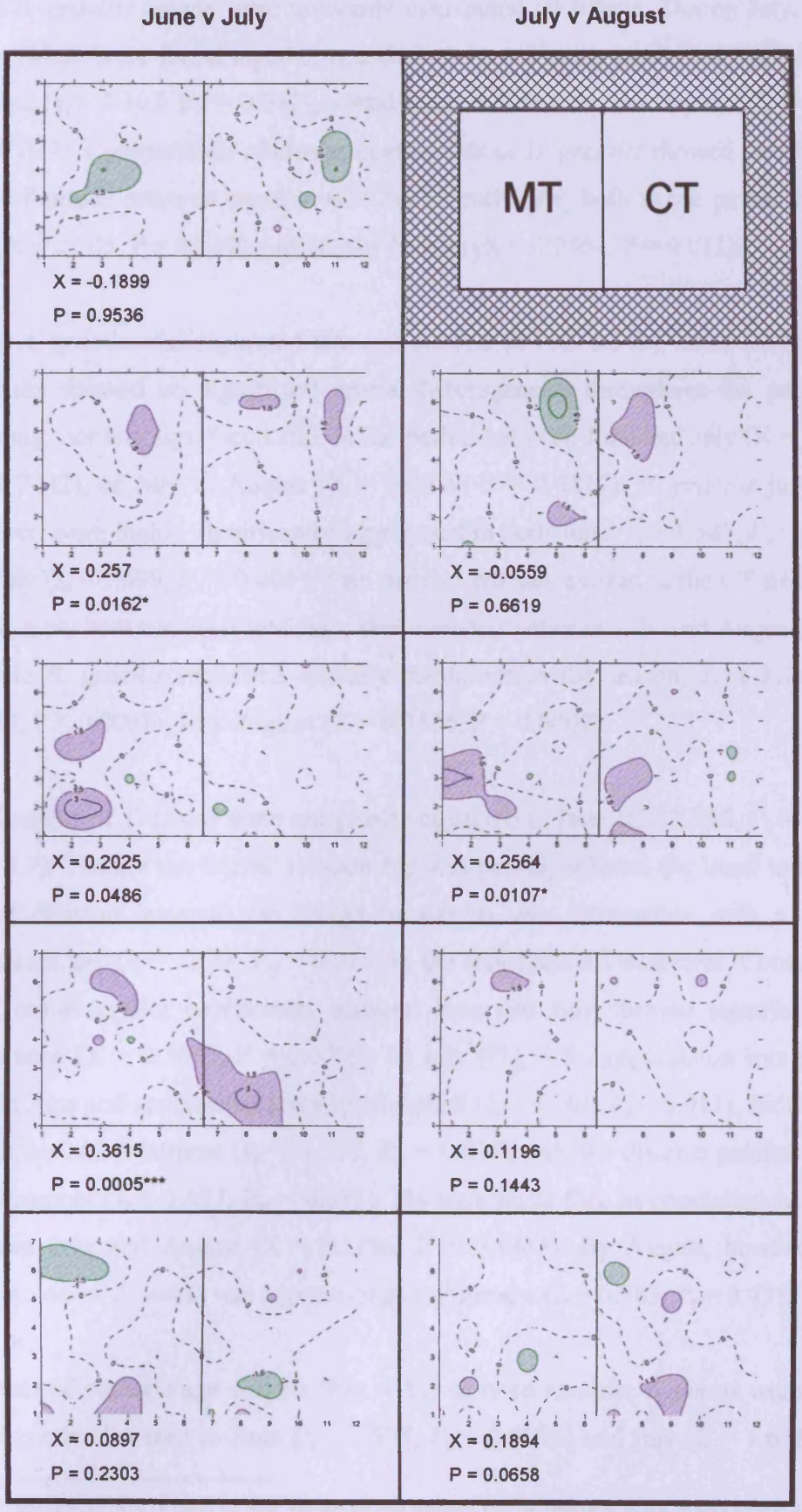
Fig. 4.10 (p90) Aphid, collembola and spider temporal co-occurence plots. Plum-green plots of the temporal co-occurrence of each species or species complex, representing the temporal flux of each species between sampling occasions.


Aphids
Collembola
B. gracilis
T. tenuis
Erigone spp.
I_a = 1.644, P_a = 0.0074*
v_j = -1.683, P_vj = 0.0055*
v_j =1.792, P_vj = 0.0018**
X = 0.650, P = <0.0001***
I_a = 2.446, P_a = 0.0002***
v_j = -2.3, P_vj < 0.0001***
v_j = 2.125, P_vj = 0.0002***
X = -0.0187, P = 0.5631
X = 0.1776, P = 0.0611
I_a = 1.309, P_a = 0.0682
v_j = -1.31, P_vj = 0.0622
v_j = 1.288, P_vj = 0.0645
X = 0.302, P = 0.0044**
X = 0.2155, P = 0.0319
X = 0.035, P = 0.3864
I_a = 1.365, P_a = 0.0478
v_j = -1.33, P_vj = 0.0151*
v_j = 1.497, P_vj = 0.057
X = 0.0404, P = 0.3583
X = 0.2161, P = 0.0319
X = 0.035, P = 0.3766
X = -0.0168, P = 0.5631
I_a = 1.16, P_a = 0.1522
v_j = -1.162, P_vj = 0.1626
v_j = 1.159, P_vj = 0.1614
Aphids
Collembola
B. gracilis
T. tenuis


Aphids
Collembola
B. gracilis
T. tenuis
Erigone spp.
I_a = 1.08, P_a = 0.2532
V_j = -1.083, P_{vj} = 0.2517
v_i = 1.035, P_{vi} = 0.335
I_a = 1.458, P_a = 0.026
v_j = -1.412, P_{vj} = 0.0365
v_i = 1.447, P_{vi} = 0.0282
X = 0.1053, P = 0.2154
I_a = 1.474, P_a = 0.027
v_j = -1.52, P_{vj} = 0.0292
v_i = 1.392, P_{vi} = 0.0124*
X = 0.112, P = 0.1159
X = 0.3621, P = 0.0003***
I_a = 1.62, P_a = 0.0111*
v_j = -1.518, P_{vj} = 0.0189*
v_i = 1.457, P_{vi} = 0.0121*
X = 0.04, P = 0.3764
X = 0.2392, P = 0.0171*
X = 0.3483, P = 0.0015**
I_a = 1.395, P_a = 0.0377
v_j = -1.299, P_{vj} = 0.0347
v_i = 1.408, P_{vi} = 0.0726
X = 0.1005, P = 0.2011
Aphids
X = -0.1292, P = 0.8357
Collembola
X = 0.0021, P = 0.4894
B. gracilis
X = 0.2791, P = 0.0095*
T. tenuis


Aphids
Collembola
B. gracilis
T. tenuis
Erigone spp.
I_a = 1.08, P_a = 0.2532
V_j = -1.083, P_{vj} = 0.2517
v_i = 1.035, P_w = 0.335
I_a = 1.458, P_a = 0.026
v_j = -1.412, P_{vj} = 0.0365
v_i = 1.447, P_w = 0.0282
X = 0.1053, P = 0.2154
I_a = 1.474, P_a = 0.027
v_j = -1.52, P_{vj} = 0.0292
v_i = 1.392, P_w = 0.0124*
X = 0.112, P = 0.1159
X = 0.3621, P = 0.0003***
I_a = 1.62, P_a = 0.0111*
v_j = -1.518, P_{vj} = 0.0189*
v_i = 1.457, P_w = 0.0121*
X = 0.04, P = 0.3764
X = 0.2392, P = 0.0171*
X = 0.3483, P = 0.0015**
I_a = 1.395, P_a = 0.0377
v_j = -1.299, P_{vj} = 0.0347
v_i = 1.408, P_w = 0.0726
X = 0.1005, P = 0.2011
Aphids
X = -0.1292, P = 0.8357
Collembola
X = 0.0021, P = 0.4894
B. gracilis
X = 0.2791, P = 0.0095*
T. tenuis


Collembola
B. gracilis
T. tenuis
Erigone spp.
I_a = 1.063, P_a = 0.2893
v_j = -1.081, P_vj = 0.2634
v_i = 1.04, P_vi = 0.3268
I_a = 1.063, P_a = 0.2693
v_j = -1.143, P_vj = 0.3226
v_i = 1.043, P_vi = 0.1792
X = 0.2781, P = 0.0126*
I_a = 0.983, P_a = 0.4319
v_j = -0.986, P_vj = 0.5115
v_i = 0.956, P_vi = 0.4277
X = 0.2683, P = 0.0214*
X = 0.3904, P = 0.0018**
I_a = 0.897, P_a = 0.6834
v_j = -0.897, P_vj = 0.5318
v_i = 0.958, P_vi = 0.6913
X = 0.2473, P = 0.0178*
X = 0.3904, P = 0.0018**
X = 0.4452, P < 0.0001***
Collembola
B. gracilis
T. tenuis


June v July
July v August
MT
CT
Aphids
X = -0.1899
P = 0.9536
Collembola
X = 0.257
P = 0.0162*
X = -0.0559
P = 0.6619
B. gracilis
X = 0.2025
P = 0.0486
X = 0.2564
P = 0.0107*
T. tenuis
X = 0.3615
P = 0.0005***
X = 0.1196
P = 0.1443
Erigone spp.
X = 0.0897
P = 0.2303
X = 0.1894
P = 0.0658

4.4.4. Spatial patterning and temporal flux of spiders

Total *B. gracilis* counts were randomly distributed all season. During July, when low v_i values were found together (i.e. they clustered into a gap[9]) in the MT half of the field (v_i = 1.392, P_{vi} = 0.012), overall their distribution was random (I_a = 1.474, P_a = 0.027). Comparisons of cluster coefficients of *B. gracilis* showed the changes in distributions between months were consistently low; both in the period June to July (X = 2.205, P = 0.049) and July to August (X = 0.2564, P = 0.011).

When *B. gracilis* was separated into age cohorts (4.11a, 4.11b), however, analysis of adults showed no significant spatial heterogeneity throughout the period of sampling, nor any significant flux in the period between June and July (X = 0.080, P = 0.2442), or July to August (X = 0.0079, P = 0.4817). *B. gracilis* juveniles, however, were highly significantly aggregated in both June (I_a = 1.587, P_a = 0.009) and July (I_a = 1.699, P_a = 0.006). Two distinct patches existed in the CT treatment, which grew between June and July, then receded between July and August. Thus, juvenile *B. gracilis* remained spatially stable across the season; June-July (X = 0.4314, P < 0.0001), July-August (X = 0.3536, P = 0.0007).

Total counts of *T. tenuis* were marginally clustered in June (I_a = 1.365, P_a = 0.048) (Fig. 4.7). Though the overall relationship was non-significant, the trend towards a spatial division between the tillage treatments was discernable with a single, significant gap (v_j = -1.33, P_{vj} = 0.015) in the minimum till treatment. Comparison of *T. tenuis* cluster coefficients between June and July showed significant co-occurrence (X = 0.3615, P = 0.0005). In July (Fig. 4.8), aggregation was greater than in June and statistically significant overall (I_a = 1.161, P_a = 0.011), for a single gap in the MT treatment (v_j = -1.518, P_{vj} = 0.012) and two discrete patches in the CT treatment (v_i = 1.457, P_{vi} = 0.012). No significant flux in population occurred between July and August (X = 0.1196, P = 0.1443). By August, however, the distribution of *T. tenuis* was approaching randomness (I_a = 0.983, P_a = 0.4319).

Analysis of separate age cohorts (Fig. 4.11) showed juvenile *T. tenuis* were again significantly clustered in June (I_a = 1.595, P_a = 0.0092) and July (I_a = 1.638, P_a =

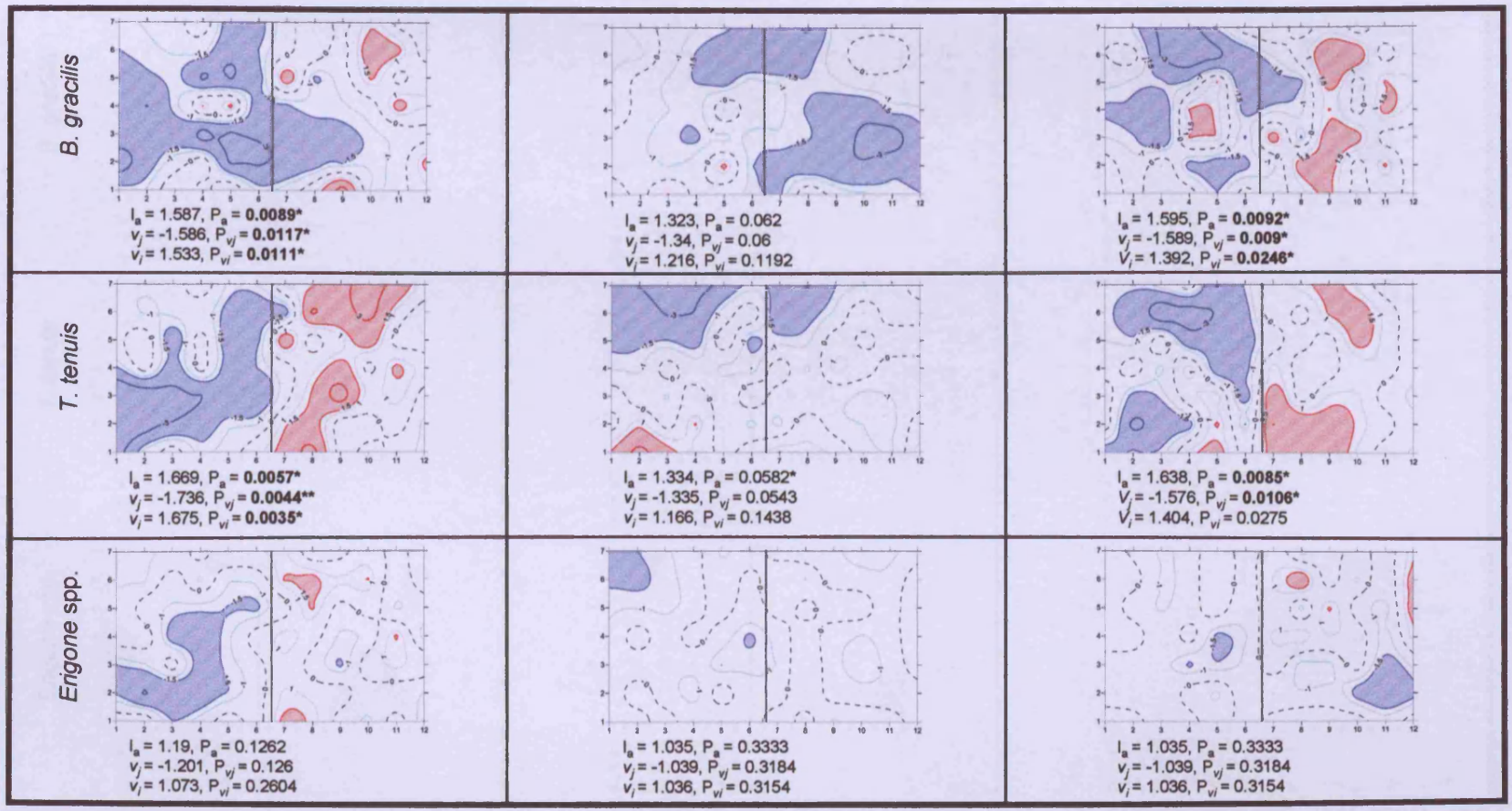
[9] Note that it is similarly high or low values of the indices (v_j for patches, v_i for gaps) which are referred to as being 'clustered', rather than actual individuals. Thus, counterintuitively, sampling points may be referred to as 'clustering into gaps'.

0.0085), with patches present in the CT treatment. Adults, however, showed no aggregation in June (I_a = 0.883, P_a = 0.7213) and tended towards a regular arrangement (disaggregation) in July (I_a = 0.8, P_a = 0.9301). Between June and July, juvenile *T. tenuis* showed significant spatial stability (X = 0.4054, P < 0.0001), but co-occurrence between July and August was apparently random (X = 0.0379, P = 0.3604). There was no pattern in these temporal shifts for adults (X = 0.0459, P = 0.352). By August, in keeping with every cohort of each species, neither adults (I_a = 0.875, P_a = 0.7386) nor juveniles (I_a = 1.035, P_a = 0.3333) were significantly aggregated.

No significant spatial patterns were evident for total counts of *Erigone* spp., nor did clustering occur in either age cohort (Fig. 4.11a,4.11b). However, among the age cohorts the trend was the opposite of that seen in the linyphiines (i.e. *B. gracilis* and *T. tenuis*) adults showed a trend towards aggregation, while juveniles were randomly arranged but with a trend towards disaggregation (Fig. 4.11a, 4.11b).

Fig. 4.11 (overleaf) SADIE red-blue plots and associated indices and their probabilities for the clustering of the spider populations Juveniles (4.11a) and adults only 4.11b).

(a)


June
July
August
B. gracilis
I_a = 1.587, P_a = **0.0089***
v_j = -1.586, P_{vj} = **0.0117***
v_i = 1.533, P_{vi} = **0.0111***
I_a = 1.323, P_a = 0.062
v_j = -1.34, P_{vj} = 0.06
v_i = 1.216, P_{vi} = 0.1192
I_a = 1.595, P_a = **0.0092***
v_j = -1.589, P_{vj} = **0.009***
V_i = 1.392, P_{vi} = **0.0246***
T. tenuis
I_a = 1.669, P_a = **0.0057***
v_j = -1.736, P_{vj} = **0.0044****
v_i = 1.675, P_{vi} = **0.0035***
I_a = 1.334, P_a = 0.0582*
v_j = -1.335, P_{vj} = 0.0543
v_j = 1.166, P_{vi} = 0.1438
I_a = 1.638, P_a = **0.0085***
V_j = -1.576, P_{vj} = **0.0106***
V_i = 1.404, P_{vi} = 0.0275
Erigone spp.
I_a = 1.19, P_a = 0.1262
v_j = -1.201, P_{vj} = 0.126
v_i = 1.073, P_{vi} = 0.2604
I_a = 1.035, P_a = 0.3333
v_j = -1.039, P_{vj} = 0.3184
v_i = 1.036, P_{vi} = 0.3154
I_a = 1.035, P_a = 0.3333
v_j = -1.039, P_{vj} = 0.3184
v_i = 1.036, P_{vi} = 0.3154

(b)

June
July
August
B. gracilis
T. tenuis
Erigone spp.
I_a = 0.958, P_a = 0.5087
v_j = -0.935, P_{vj} = 0.5274
v_j = 0.894, P_{vj} = 0.7236
I_a = 0.97, P_a = 0.4796
v_j = -1.005, P_{vj} = 0.3888
v_j = 0.972, P_{vj} = 0.4691
I_a = 0.952, P_a = 0.5244
v_j = -0.962, P_{vj} = 0.51
v_j = 0.857, P_{vj} = 0.8408
I_a = 0.883, P_a = 0.7213
v_j = -0.876, P_{vj} = 0.7369
v_j = 0.891, P_{vj} = 0.7123
I_a = 0.8, P_a = 0.9301
v_j = -0.796, P_{vj} = 0.9442
v_j = 0.824, P_{vj} = 0.8954
I_a = 0.875, P_a = 0.7386
v_j = -0.877, P_{vj} = 0.7525
v_j = 0.861, P_{vj} = 0.8289
I_a = 1.141, P_a = 0.1765
v_j = -1.139, P_{vj} = 0.1895
v_j = 1.18, P_{vj} = 0.1495
I_a = 0.972, P_a = 0.4813
v_j = -0.981, P_{vj} = 0.4448
v_j = 0.96, P_{vj} = 0.5049
I_a = 1.337, P_a = 0.0.0577
v_j = -1.342, P_{vj} = 0.0587
v_j = 1.259, P_{vj} = 0.087

4.4.5. Linyphiid co-occurrence with conspecific juveniles

Mapping of the co-occurrence between linyphiid adults and their conspecific young differed among species (Table 4.2) *Tenuiphantes tenuis* adults significantly overlapped with their juveniles in June (X= 0.2976, P = 0.005), then tended towards disassociation in July (X = -0.1363, P = 0.8815), before returning to significant association in August (X = 0.4465, P < 0.0001). *Bathyphantes gracilis* adults did not aggregate with their juveniles at any point during the year.

Inter age-cohort co-occurrence	June		July		August	
	X	P	X	P	X	P
B. gracilis AD v JUV	0.3540	0.3828	0.1915	0.9561	0.0068	0.516
Erigone spp. AD v JUV	0.2316	0.0566	0.1033	0.1928	0.1172	0.8424
T. tenuis AD v JUV	0.2976	**0.005***	0.1363	0.8815	0.4465	**0.0001*****

Table 4.2. The overall spatial co-occurrence between each linyphiid species' two age cohorts. JUV contains the non-breeding population i.e. juveniles and sub-adults.

4.4.6. Numerical response of spiders to prey clustering

A significant numerical response was apparent early in the season by *B. gracilis*. This spider's population growth between June and July was significantly positively related to the aphid cluster coefficients from the June samples ($F_{TOT} = 6.173_{77,1}$, P = 0.015). Separate analyses by age category revealed this was mainly due to increases in juveniles ($F_{JUV} = 4.858_{77,1}$, P=0.031); if adults only were considered, no significant response was found ($F_{AD} = 1.021_{77,1}$, P = 0.316). Between July and August, *B. gracilis*' population showed a marginally non-significantly negative response to collembola cluster coefficients ($F_{TOT} = 5.11_{77,1}$, P = 0.026).

Population growth of *T. tenuis* between June and July was significantly related to the June cluster coefficients of both aphids ($F_{TOT77,1} = 5.234$, P = 0.025) and collembola ($F_{TOT77,1} = 5.294$, P = 0.0241). However, as with *B. gracilis*, when similar analyses were run by spider age category, only juveniles showed a significant response to both aphids ($F_{JUV7,1} = 5.444_{7}$, P = 0.005) and collembola ($F_{JUV77,1} = 5.661$, P = 0.020).

The only significant relationship between *Erigone* spp. and the spatial clustering of aphids or collembola, was a negative response by *Erigone* spp. adults to the July

cluster coefficients of aphids ($F_{AD77,1}$ = 8.657, P = 0.005). Full results are shown in Table 4.3.

Predator cohort	Prey	Prey date	Predator growth period	Slope	P	Multiple r^2	$F_{1,78}$
B. gracilis TOTAL	Aphid	June	June-July	0.368	0.015*	0.073	6.173
	Aphid	July	July-Aug	-0.047	0.872	<0.001	0.026
B. gracilis ADULT	Aphid	June	June-July	0.076	0.316	0.013	1.021
	Aphid	July	July-Aug	-0.113	0.414	0.009	0.674
B. gracilis JUVENILE	Aphid	June	June-July	0.292	0.031*	0.059	4.858
	Aphid	July	July-Aug	0.131	0.611	0.611	0.261
B. gracilis TOTAL	Collembola	June	June-July	0.183	0.124	0.030	2.413
	Collembola	July	July-Aug	-0.472	0.027*	0.062	5.114
B. gracilis ADULT	Collembola	June	June-July	-0.015	0.801	<0.001	0.064
	Collembola	July	July-Aug	-0.091	0.377	0.010	0.790
B. gracilis JUVENILE	Collembola	June	June-July	0.198	0.0603	0.045	3.635
	Collembola	July	July-Aug	-0.264	0.167	0.024	1.945
T. tenuis TOTAL	Aphid	June	June-July	0.553	0.025*	0.063	5.234
	Aphid	July	July-Aug	-0.053	0.933	<0.001	0.007
T. tenuis ADULT	Aphid	June	June-July	-0.099	0.339	0.012	0.925
	Aphid	July	July-Aug	0.161	0.451	0.007	0.575
T. tenuis JUVENILE	Aphid	June	June-July	0.652	0.005**	0.099	8.544
	Aphid	July	July-Aug	-0.214	0.717	0.002	0.132
T. tenuis TOTAL	Collembola	June	June-July	0.432	0.024*	0.063	5.294
	Collembola	July	July-Aug	-0.578	0.220	0.019	1.531
T. tenuis ADULT	Collembola	June	June-July	0.013	0.875	<0.001	0.025
	Collembola	July	July-Aug	-0.001	0.994	<0.001	<0.001
T. tenuis JUVENILE	Collembola	June	June-July	0.419	0.020*	0.068	5.661
	Collembola	July	July-Aug	-0.577	0.187	0.022	1.770
Erigone spp. TOTAL	Aphid	June	June-July	-0.046	0.544	0.005	0.371
	Aphid	July	July-Aug	-0.047	0.734	0.001	0.117
Erigone spp. ADULT	Aphid	June	June-July	-0.002	0.973	<0.001	0.002
	Aphid	July	July-Aug	-0.151	0.004**	0.010	8.657
Erigone spp. JUVENILE	Aphid	June	June-July	-0.045	0.5084	0.006	0.442
	Aphid	July	July-Aug	0.104	0.442	0.008	0.597
Erigone spp. TOTAL	Collembola	June	June-July	-0.033	0.579	0.004	0.311
	Collembola	July	July-Aug	-0.010	0.924	<0.001	0.009
Erigone spp. ADULT	Collembola	June	June-July	-0.058	0.088	0.039	2.981
	Collembola	July	July-Aug	-0.025	0.528	0.005	0.401
Erigone spp. JUVENILE	Collembola	June	June-July	0.026	0.627	0.003	0.239
	Collembola	July	July-Aug	0.016	0.877	<0.001	0.024

Table 4.3. Linear regressions to compare the local response of each spider to the local SADIE cluster coefficients of each of the first-order prey (aphids and collembola) at the start of the period for which the population change was measured.

4.4.7. Prey response to predator aggregation

Collembola did not significantly respond to the clustering of any predator species. Average aphid counts declined between June and July (Fig. 4.5). The magnitude of local declines in aphid abundance, however, were positively related to the corresponding local cluster coefficients of the following: *B. gracilis* juveniles ($F_{1,78}$ = 8.582, P = 0.0045), the total *T. tenuis* population ($F_{1,78}$ = 5.655, P = 0.0199), adults of *Erigone* spp. alone ($F_{1,78}$ = 6.362, P = 0.0137) and the whole *Erigone* spp. population ($F_{1,78}$ = 7.464, P = 0.0078). No changes in either prey species between July and August were significantly related to clustering of prey in July. Full results are shown in Table 4.4.

Predator	Prey	Growth period	Prey date	Slope	P	Multiple r^2	$F_{1,78}$
B. gracilis ADULT	Aphid	June-July	June	0.111	0.370	0.010	0.814
B. gracilis JUVENILE		June-July	June	-0.204	0.005**	0.099	8.582
B. gracilis TOTAL		June-July	June	-0.108	0.203	0.021	1.649
B. gracilis ADULT	Collembola	June-July	June	0.080	0.639	0.004	0.221
B. gracilis JUVENILE		June-July	June	-0.174	0.082.	0.038	3.115
B. gracilis TOTAL		June-July	June	-0.162	0.164	0.025	1.972
B. gracilis ADULT		July-Aug	July	-0.067	0.756	0.001	0.097
B. gracilis JUVENILE		July-Aug	July	-0.092	0.388	0.001	0.752
B. gracilis TOTAL		July-Aug	July	-0.258	0.044*	0.051	4.176
T. tenuis ADULT	Aphid	June-July	June	-0.239	0.054.	0.047	3.832
T. tenuis JUVENILE		June-July	June	-0.132	0.060.	0.045	3.655
T. tenuis TOTAL		June-July	June	-0.167	0.020*	0.068	5.655
T. tenuis ADULT	Collembola	June-July	June	-0.177	0.302	0.014	1.082
T. tenuis JUVENILE		June-July	June	-0.039	0.693	0.002	0.157
T. tenuis TOTAL		June-July	June	-0.149	0.133	0.029	2.304
T. tenuis ADULT		July-Aug	July	0.052	0.851	<0.001	0.035
T. tenuis JUVENILE		July-Aug	July	-0.085	0.509	0.006	0.441
T. tenuis TOTAL		July-Aug	July	-0.076	0.545	0.005	
Erigone spp. ADULT	Aphid	June-July	June	-0.307	0.013*	0.075	6.36
Erigone spp. JUVENILE		June-July	June	-0.065	0.548	0.004	0.365
Erigone spp. TOTAL		June-July	June	-0.283	0.008**	0.087	7.464
Erigone spp. ADULT	Collembola	June-July	June	-0.073	0.675	0.003	0.177
Erigone spp. JUVENILE		June-July	June	-0.190	0.196	0.021	1.701
Erigone spp. TOTAL		June-July	June	-0.377	0.010	0.084	7.051
Erigone spp. ADULT		July-Aug	July	0.274	0.209	0.020	1.608
Erigone spp. JUVENILE		July-Aug	July	-0.073	0.640	0.003	0.221
Erigone spp. TOTAL		July-Aug	July	0.033	0.836	<0.001	0.043

Table 4.4. Linear regressions to compare the local response of each prey species to the local SADIE cluster coefficients of each of the spiders (aphids and collembola) at the start of the period for which the population change was measured.

4.5. Discussion

4.5.1. Environmental factors, tillage and crop yield

Overall, the crop yield under CT was greater than that under MT. The yield was highest where the ground was devoid of any cover. It is likely that the effects of ploughing in the residues (i.e. organic matter) from the previous crop boosted the amount of nutrients available to the crop (Rieger *et al.* 2008), while simultaneously providing a higher moisture content in the top layer (soil moisture is inversely proportional to soil hardness). Furthermore, the harvesting of the previous crop resulted in uneven deposition of the organic matter (caused by blockages in the combine harvester) (Bell, pers. comm.). On the CT side of the field this would have been ploughed into the soil irregularly, but under MT been evenly distributed by the cultivation. Algae was only apparent in significant quantities on the CT soil. Yields were significantly higher in the CT treatment, the best being obtained from the 'NE' corner of the field from which algae and organic matter were absent and the soil was particularly soft (i.e. moist).

4.5.2. Prey species distributions

Most previous analyses of the relationship between crop yield and herbivore density have found a negative relationship (Lang 2003, Östman *et al.* 2003, Cardinale *et al.* 2003, Snyder *et al.* 2006). Contrary to these studies, however, yield was in this case found to be positively related to the total aphid density. Aphids showed high levels of spatial aggregation at the start of the season (flowering), but this aggregation proved highly unstable. By the time the wheat had ripened (mid July), their distribution was random and by harvest (early August), sampled populations had dropped to zero. Such transience is apparent in other studies of aphid distributions. Some degree of non-random spatial arrangement persists, but occurs at increasingly smaller scales as the season progresses (Winder *et al.* 1999. Fievet *et al.* 2007). The movement of aphids at the field scale is driven by a number of interacting causes including parasitism and predation (Schmidt *et al.* 2003, Traugott *et al.* 2008), weather (Mann *et al.* 1995, von Berg *et al.* 2008), and declining host suitability (Watt & Dixon, 1981, Dixon & Glen 1971).

As aphids migrate into the crop, plant suitability is likely to initially be the most influential factor in the aphids' spatial distribution. It seemed therefore, that the initial spatial distributions of aphids were driven by differences in host suitability (Duffield *et al.* 1997). This suitability arises from higher levels of fixation of nutrients from the soil, differences in which were seemingly a response to the different tillage regimes (Fig. 4.4). Localities which eventually yielded higher than average amounts of grain were mostly found in the CT treatment (Figures 4.7, 4.8 and 4.9). Thus, crop yield was positively associated with higher than average aphid counts in June. However, by July, despite being more numerous than in June, the positive relationship between their abundance and wheat yield no longer remained. This was likely due to a combination of the aphids response to maturing plants, crowding and predation (Dixon 1999). Crowding and/or later stages of plant growth induce the aphids to produce more asexual alates (winged morphs) capable of colonising new host plants (Watt & Dixon 1981), meaning that while more plants are colonised, colony size drops (Fievet *et al.* 2007). The subsequent precipitous decline of aphid abundance is therefore not unsurprising, especially given the low rainfall during this season.

The economic thresholds for damage by aphids are thus lower when the crop is younger and growing more rapidly (Larsson 2005), so those tillers that attracted aphids at flowering (June) may have suffered disproportionately greater damage than they would have from the same levels of infestation later in the year (Entwistle & Dixon 1987). Additionally, even at low densities the aphids may prove damaging as vectors of disease. Yellow Barley Dwarf Virus, for example, reduces yield, then propagates by means of reducing the fitness of aphids feeding on infected plants, thus encouraging more alates, which spread the virus (Fieberg *et al.* 2004). This is only usually a problem with Autumn infestations, however.

Higher counts of epigeic isotomid collembola were associated with the CT regime than with the MT regime. This is contrary to many studies, the majority of which have shown that tillage lowers the abundance of these detritivores, though mostly without diminishing species richness (Brennan *et al.* 2006 & refs therein). Nevertheless, DeRuiter *et al.* (1993), while comparing the rates of N mineralization between CT and MT regimes, found higher abundances of microarthropods in the top 10 cm of soil in CT compared to the MT regime similar to that carried out for

this study (disc cultivation of the top 10cm of soil). The soil fauna, aphids and crop yield were also likely (Eisenhaur *et al.* 2010) may also alter the rates at which N becomes available to the crop (Ke & Scheu 2008), possibly resulting in synergistic (i.e. non-additive) alterations in crop yield. Ultimately, though, an increased availability of carbon-rich organic matter in the top layer of soil will increase collembola populations. Whether or not this occurs depends both on the nutritional inputs to the upper soil horizon and the tillage regime. Moreover, such inputs also alter the complexity of the microhabitat at the soil surface independently of nutrient availability. This last point was demonstrated by Birkhofer *et al.* (2008), who showed a decline in the activity-density of carabids and cursorial spiders in plots provided with inedible matter which increased microhabitat complexity.

4.5.3. Predator-prey interactions

Significantly higher populations of total spiders were found in the conventional tillage treatment than in the minimum tillage regime, contradicting the findings of Holland *et al.* (2003) who, in a study of the effects of tillage on a range of arthropods, found Araneae to be less numerous in ploughed plots. However, the higher spider populations in the CT treatment in the present study seem to be a response to the higher numbers of potential prey. Linyphiid predators are more likely to persist at patches of high prey abundance, indicating a preference for such sites (Samu *et al.* 1996b). Furthermore, they are also more likely to locate their webs at such sites (Harwood *et al.* 2003). These investigators (Harwood *et al.* 2003) sampled the availability of prey surrounding web-owning and non-web-owning *Tenuiphantes tenuis* and populations of *Erigone* spp., at sites that were paired on the basis of their structural complexity. They found higher abundances of prey where the spiders chose to make webs. Collembola were more common at *Erigone* spp. websites, while aphids were particularly high at those of *T. tenuis* (Harwood *et al.* 2003).

In the present study, the broad trend in the abundance of the whole populations (i.e. adults plus non-reproductives) of linyphiid spiders *Bathyphantes gracilis*, *T. tenuis* and *Erigone* spp. was that of a lagged response to aggregations of their prey. In all three species their rates of aggregation, relative to the range they showed over the year, were intermediate in June, most aggregated in July and the least so in August (Figures 4.7, 4.8 and 4.9). Generalists are thought to be able to maintain their

populations on non-pest prey, allowing them to respond to pests as their numbers fluctuate, hence lowering or retarding peak pest populations (Edwards *et al.* 1979, Settle *et al.* 1996, Symondson *et al.* 2002a). Increases in collembola densities may or may not translate to increased natural enemy abundance (Bell *et al.* 2008). However, even where this positive relationship is the case, aphids may indirectly benefit due to prey switching to subsequently more numerous detritivores (Birkhofer *et al.* 2008).

Likely contamination of linyphiid predators as a result of the sampling method employed precluded a direct assessment of predation levels. Thus, the relationship between prey abundance (expressed as SADIE cluster coefficients) and subsequent local predator population changes was examined as a proxy of this, to attempt to elucidate the significance of particular predator-prey trophic interactions. High abundances of aphids and collembola were found in the same part of the field (i.e. CT regime), which was assumed to have been a response to interactive differences in plant quality and soil properties (Figures 4.7, 4.8 and 4.9). Moreover, linyphiids (at least in the case of *T. tenuis* and *B. gracilis*) seemed to respond to higher rates of prey.

The relationships between the rates of decline in aphids and a number of the linyphiid predators suggested that these species were closely coupled. *B. gracilis* adults were at no point associated with aphids so seemingly played little part in aphid decline. However, *B. gracilis* juveniles showed significant spatial co-occurrence with aphids in June. Subsequently, aphids significantly declined in relation to this proximity, while *B. gracilis* juveniles concomitantly rose, suggesting that at this stage in the season this trophic interaction was tightly coupled. All of these common linyphiid species show rapid gestation of egg sacs of between 13-18 days (Thorbek *et al.* 2003), meaning that potentially, even an association with late-stage juveniles may have responded by increasing birth rates and their offspring having time to mature between sampling occasions, Therefore constituting a numerical response of these spiders. Dispersal, of both juveniles or adults, may have a positive effect on population levels of linyphiids. However, Topping & Sunderland (1998) found that in *T. tenuis* at least, immigration resulted in no net losses or gains to overall populations.

Both total populations of and the two age cohorts of *T. tenuis* were positively associated with aphids in June (Table 4.3). *T. tenuis* growth between June and July was positively associated with the magnitude of the clustering of aphids in June. Moreover, a concomitant decline in aphids was associated with higher local abundance of *T. tenuis* in June. Only juvenile *T. tenuis* responded positively (between June and July) to increased clustering of collembola in June, but were not associated with simultaneous collembola population reductions, suggesting that juvenile *T. tenuis* were not consuming collembola to the degree which they did aphids. During July and August, *T. tenuis* maintained a significant spatial aggregation with collembola, but no significant numerical responses (positive or negative), were apparent either in terms of increases in predator numbers or reductions in prey.

It seems likely that the spatiotemporal patterns exhibited by *T. tenuis* are a consequence of these predators tracking mainly aphid prey, and of subsequent reproductive benefits to the predators (Winder *et al.* 2001). This is supported by evidence showing that Aphididae are the principal component of the *T. tenuis* diet in Italian Rye grass, while collembola are a secondary component (Alderweireldt 1994b). It also seems apparent from the present study that the population dynamics of *T. tenuis* had no significant negative effect on collembola abundance. Taken together, *T. tenuis'* spatial relationships with collembola and aphids in this study, conform to those described by Harwood *et al.* (2005) who showed that *T. tenuis* consume aphids independently of the availability of isotomid collembola.

Erigone spp. were expected to track abundance of prey, in particular collembola (Harwood et al. 2003, Alderweireldt 1994b). However, neither age cohort showed any spatial association with aphids in June. *Erigone* spp. adults were significantly associated with collembola in June, and were marginally associated with aphids in July. Higher cluster coefficients of both adults and total *Erigone* spp. were associated with negative growth rates of the aphids between June and July.

Spatial relationships among predators must also be considered along with those between predators and prey, as there is likely to be a certain level of intra-and inter-specific competition for resources Fig. 4.10 and Figures 4.7, 4.8, 4.9 show the spatial relationships that will contribute to these forces. Furthermore, these species

are intraguild prey of carabids (Chapter 3) and so will also experience apparent competition (i.e. competition for predator-free space (Holt & Lawton 1994, Chapter 2). However, the intraspecific spatial interactions are just as likely to be driven by the sites that adults choose to lay eggs. At the whole-field scale (i.e. encompassing both tillage treatments) *T. tenuis* adults were significantly aggregated with their juveniles in June and randomly distributed in July. This was followed by a second period of aggregation in August. These points of aggregation corresponded to population increases. The similarity of cluster coefficients between all months for *B. gracilis* suggested that populations of this species were, relative to the other linyphiid species, highly stable. *Bathyphantes gracilis* adults and juveniles were randomly distributed in relation to each other for the whole year. The species' propensity to migrate along the ground, rather than aerially (Alderweireldt 1989) might have contributed to this stability. The *Erigone* spp. adults and juveniles were marginally aggregated in June, then randomly spatially distributed thereafter.

4.5.4. Linking predation and predator function to spatial patterns

A number of studies have found similar associations to those found in the present study between generalist predators and pest species in winter wheat. Winder *et al.* (2005), for example, also showed that increased activity-density of the carabid *Poecilus cupreus* corresponded to lower aphid abundance, with the patterns of both predators and prey tending towards randomness as the season progressed,. Moreover, a number of these studies have simultaneously estimated levels of predation with spatiotemporal analyses of invertebrate distributions at the field scale. Winder *et al.* (2005), showed similar dynamics, but used ELISA to explicitly test whether two carabids, *Pterostichus melanarius* and *P. madidus* were spatially associated with aphids through predation. They found an association between aphid clustering and ELISA positives early in the season while the aphid population was increasing, but no association during the subsequent aphid population decline. Importantly, these interactions were found at a similar scale to the present study (Bohan *et al.* 2000). Bell *et al.* (2010) provide additional evidence that spatial proximity relates to predation by demonstrating a positive relationship between predator-prey proximity (using measures of SADIE co-occurrence) and the likelihood of predation between *P. melanarius* and earthworms.

Web-owning *T. tenuis*, and therefore probably those individuals of *B. gracilis* able to defend a web-site, consume more aphids than non web-owning individuals (Harwood *et al.* 2004). The earlier arrival of a competitor for similar prey may prevent the latecoming individual from building its web at an optimal height (Herberstein 1998, Harwood & Obrycki 2007). Thus, in many cases, such priority effects may affect the partitioning, in the horizontal plane, of niche space between *B. gracilis* and *T. tenuis*. Nevertheless, the two species occupy significantly different niches along the vertical spatial axis (Sunderland *et al.* 1986, Alderweireldt 1994b). Furthermore, this segregation was found to increase as the season progressed (Sunderland *et al.* 1986) as a consequence of *B. gracilis* placing their webs at increasing heights from the soil surface. In the results presented here, the two species' horizontal spatial overlap increased as the season progressed, presumably in response to depleted aphid prey. It is possible, therefore, that rising heights of *B. gracilis'* webs may be a response to increases in horizontal spatial niche overlap.

The sampling methods employed in this experiment, while providing efficient estimates of populations for all the species involved in this analysis (Topping & Sunderland 1998, Elliot *et al.* 2006), make it impossible to determine where the linyphiines sampled (*B. gracilis* and *T. tenuis*) existed along this gradient from web-dependence to active hunting (Alderweireldt 1994a, 1994b, Harwood *et al.* 2001, 2003). This inability to determine predator function means that caution must be exercised in any interpretation of the observed patterns of co-occurrence. *Tenuiphantes tenuis*, for example, displays differential rates of airborne migration between sexes and life stages at different times of the season, with activity rates of males dependent on whether or not they have mated (Topping & Sunderland 1998). Thus, abundance does not reflect activity, but neither does the amount of activity reflect the amount of time spent hunting for prey. Individual-based, spatially explicit models (Rosenheim & Corbett 2003) used to investigate the role of predator function in predicting trophic interactions, predicted that sit-and-wait predators would be more likely to consume mobile prey while mobile predators would be more likely to consume sedentary prey, leading to so-called 'trophic switches'.

4.5.5. Conclusions

A number of factors were expected to drive the spatial patterns of the arthropods in this study. Here, aphid abundance was expected to reduce plant yield but, unexpectedly, aphid abundance increased with yield, particularly when the wheat was in a growth stage. As the year progressed, however, clusters of aphids were seen to fragment, as evidenced by the overall clustering coefficients reaching values (I_a) closer to 1 (random) (compare Fig. 4.7 and 4.8). Aphid populations were spatially unstable, declining between June and July, before becoming absent in August (Fig. 4.9). This process is likely to be caused by a number of factors including declining plant suitability, climate and parasitoids. However, the relationships described between aphids and linyphiids, in particular the linyphiines *T. tenuis* and *B. gracilis*, suggest that these natural enemies comprised a significant element of this decline. Furthermore, the relative lack of such relationships between this seemed to be the case despite the distraction of alternative prey.

5. Spatiotemporal analysis of intraguild predation by two generalist carabids, *Pterostichus melanarius* and *P. madidus*.

Abstract

While crop pests are conventionally managed with artificial pesticides, it is also important to understand the relationships between pests and their natural enemies. Natural enemies are a vital source of pest control, but interactions between them such as intraguild predation (IGP), can potentially increase the risk of pest outbreaks. Predation by the carabids *Pterostichus melanarius* and *P. madidus* on pest (aphid), non-pest (Collembola) and intraguild (adult linyphiid spiders) prey was assessed by analysing DNA from the beetles' gut-contents using PCR. Predation rates on linyphiids by the two carabids were generally low. Monte Carlo models were used to test the expectation that prey density would predict predation levels, while controlling for the beetles' circadian rhythms and interspecific differences in assay efficacy. Predation was largely density-dependent, reflecting results of similar studies. Predation on *B. gracilis* was consistently lower than expected, while *Erigone* spp. were preferred by *P. melanarius* in July and August. *T. tenuis* was never preferred, suffering less predation than expected in August. These results suggested that the likelihood of IGP substantially decreasing pest consumption was low, because when any species of spider suffered disproportionately high predation, aphid predation was also disproportionately high. This suggested spatial co-occurrence and predation may be positively related. Averaged across all the collection points, spatial proximity only predicted per capita predation rates in August. The relationships between an index of local predator abundance and subsequent prey population changes, or prey clustering versus predator responses provide evidence of dynamic linkage between species. However, few of these reationships were significant, despite the prey preferences suggested by the Monte Carlo analyses. From the perspective of biological control, the absence of significant relationships suggests a large degree of prey switching and hence weak, ephemeral trophic interactions. Where omnivory[1] such as IGP is present weak dynamic interactions are thought to stabilise food webs.

5.1. Introduction

5.1.1. Intraguild predation and stability in aphidophagous systems

According to theory based on three-species food webs (Holt & Lawton 1994) intraguild predation (IGP) (Polis *et al.* 1989, Polis & Holt 1992) has the potential to destabilise trophic interactions. Such predation is predicted to minimise the range of parameters under which both intraguild predator (IG predator) and intraguild prey (IG prey) may coexist (Daugherty *et al.* 2007, Holt & Huxel 2007, section 1.6). Thus, where an assemblage of predators exploits their shared prey resource non-additively (i.e. predation on the shared prey is greater in the presence of both IG predators and IG prey than either alone) (Losey & Denno 1998, 1999), local extinction or reduction of one of these predators results in increased prey populations (Finke & Denno 2003). Where this prey species is a pest with a high reproductive rate, this can disrupt biological control (Shurin *et al.* 2002).

Omnivory (consumption from more than one trophic level) is common in nature (Arim & Marquet 2004, Bascompte & Melian 2005). IGP is a form of omnivory (Polis & Holt 1992, Woodward & Hildrew 2001), that is especially frequent amongst assemblages of aphidophagous predators and parasitoids (Traugott & Symondson 2008, Rosenheim *et al.* 1995), whose prey are likely to aggregate in space and time because of their high growth rates and tendency only to disperse in respose to crowding (Dixon 1998).

In the majority of cases such intraguild trophic interactions do not affect biological control (Jannsen *et al.* 2006), even when the IG predator is a superior competitor for the shared prey, which is thought likely to preclude, or at least significantly diminish the probablility of, the local coexistence of both IG prey and IG predator (Holt & Polis 1996). A number of mechanisms have been proposed to solve this discordance between theory and observation. Non-linear functional responses represent switching between the most common prey by generalist predators (Abrams & Matsuda 2003, Abrams & Fung 2010). This may, in turn, act to skew the distribution of the strength of trophic interactions towards weaker links. When this occurs in food webs in which omnivory is present, stability is more likely (Emmerson & Yearsley 2004).

In addition to trophic interactions, the spatial structures of habitats are fundamental to understanding how stable cycles rather than chaos may emerge from systems that are subject to stochastic assembly (Hassell *et al.* 1991, Tilman 2004) and reciprocal dynamics between resources, prey and predators (chapter 4). Indeed, transient spatiotemporal dynamics (Tobin & Bjørnstad 2003, Bell *et al.* 2010) have been proposed as a contributing factor to stable cycling both in specialist predator-prey systems (Tobin & Bjørnstad 2003) and more generally (Hastings 2001, Keeling *et al.* 2001). Thus, the dynamic spatial heterogeneity that emerges as a consequence of enemy-victim interactions (Winder *et al.* 2001, Bohan *et al.* 2000, chapter 4), creates temporary refugia for natural enemies subject to IGP, so that provided spatial heterogeneity exists, global stability (i.e. at the field-scale in this case) may occur even when local stability is endangered by IGP.

5.1.2. Generalists as pest control agents

The role of generalist predators as agents of pest control is complementary to that of specialists (Chang & Kareiva 1999). While specialist consumers of pests (especially parasitoids) may exert more effective reductions in pest numbers (Chambers & Aikman 1988), they are often not present in sufficient numbers to do so until they have numerically responded to their prey. Generalists may therefore fulfil this role. Indeed, in the case of aphids, their exponential growth rates mean that consumption of a given number of this prey when they are at low densities is a more effective form of pest control than the same amount of predation at higher densities (Chiverton 1986). Cereal aphid such as *Sitobion avenae* and *Rhapalosiphum padi* have been documented as prey for *Pterostichus* spp. (see Harper *et al.* 2005, Winder *et al.* 2005, Lövei & Sunderland 1996, Griffiths *et al.* 2008) and linyphiid spiders (Harwood *et al.* 2003, 2005).

Pterostichus spp. can track and consume pest arthropod species (Kromp 1999, Lövei & Sunderland 1996, Symondson *et al.* 2002b). However, such effects depend on the presence of sufficient numbers of *Pterostichus* spp., along with their wide distribution in the crop. Slugs, including the pest species *Deroceras reticulatum* are also documented as being consumed by adults of both *P. melanarius* (see Symondson *et al.* 1996, Symondson *et al.* 2002a, Harper *et al.* 2005, King *et al.* unpublished data, Symondson *et al.* 2002b) and *P. madidus* (see Ayre & Port 1996, Symondson & Liddell 1993, King *et al.* unpublished data). Additionally, the

subterraneous larvae of *P. melanarius* have been shown to respond to the odour cues of both live and recently dead slugs and dipterans. As these species make up prey in the adult diet, the larvae are thus also predatory (Thomas *et al.* 2008, 2009). Dietary analysis, however, is often confined to examining carabid consumption of pests, when in reality, carabids often eat whatever they can swallow in the field (Lövei & Suderland 1996). Evidence that carabids often consume other predators comes from a number of field studies which examined the gut contents of carabids (Davies 1953, Dawson 1965, Penney 1966, Luff 1974, Sunderland 1975, Hengeveld 1980 (see Appendix X for details).

Laboratory feeding trials provide a worthwhile starting point when attempting to ascertain the feeding 'choices' predators make in the field. Such trials can give some measure of food preferences under controlled conditions. Kielty *et al.* (1999) found a number of carabids, including *P. melanarius* and *P. madidus*, preferred the aphid *Metopolophium dirhodum* when offered a choice between this and other aphid species *Brevicoryne brassicae*, *Sitobion avenae* and *Rhopalosiphum padi*. When both carabids were subsequently also provided with a choice between *M. dirhodum* and entomobryid collembola, *P. madidus* showed no preference, while *P. melanarius* preferred the aphid. However, when field-caught beetles were tested for aphid protein using ELISA (Winder *et al.* 2005) proportionally more *P. madidus* were found to have consumed aphids than *P. melanarius*. Such differences between laboratory and field studies are likely to be a function of the prey's apparency to the predator in the field, mediated by a number of factors including phenology, diel overlap (section 3.3.5.3, Chapman *et al.* 1999), and the availability of physical refuges for prey provided by environmental complexity (McKemey *et al.* 2003).

5.1.3. Spatiotemporal activity of Pterostichus madidus *and* P. melanarius *in arable crops*

The phenology of *P. melanarius and P. madidus* ensures that they are present early in the crop season when aphid numbers are low. Both these beetles are univoltine and breed in the early autumn, laying their eggs subterraneously at depths of up to 50 cm (Thomas *et al.* 2008), before taking refuge at this depth. In arable habitats, some adults also survive after harvest by taking refuge in field boundaries (Holland *et al.* 2009). Both species usually emerge between late May and early July. *P. melanarius* emerges at a more uniform rate than *P. madidus*, with the majority of

the latter surfacing in a short period during mid-June (Holland & Reynolds 2003). The emergence of *P. madidus* has been shown to be negatively affected by ploughing, irrespective of whether this took place in winter or early spring (Holland & Reynolds 2003). *P. melanarius* on the other hand, in a two-year study, has been found to suffer from ploughing in one year and benefit in the next (Holland & Reynolds 2003). The timing of ploughing is also important, Fadl *et al.* (1996) finding that emergence of *P. melanarius* was reduced where cultivation took place in spring compared to fields that were ploughed in autumn. The increased vulnerability of *P. madidus* may be a consequence of the vertical spatial niche occupied by pupae and newly emerged adults of this species, which is closer to the surface than that inhabited by *P. melanarius.* Ploughing in late spring has the greatest negative effect on carabid emergence overall, which is thought to be because the pupae reside more closely to the top of the soil column at this time, prior to emergence (Purvis & Fadl 1996, Thomas *et al.* 2008).

5.1.4. Spatial distributions

Where the spatial patterns of both *P. madidus* and *P. melanarius* have been observed at the whole farm scale, the stability of spatial aggregations of *P. madidus* both within and between seasons has been shown to be lower than *P. melanarius.* These patterns seem to mainly be a consequence of *P. melanarius'* reduced propensity to cross barriers such as hedgerows (Thomas *et al.* 1998 Holland *et al.* 2009). Mechanisms for this, however, are unclear. SADIE analysis of the aggregation patterns of *P. melanarius* showed this species persisted in broadly the same area for three years, regardless of the crop planted, on a sampling grid spaced 30 m apart spanning 64 hectares of arable land (Holland *et al.* 2009). The same analysis demonstrated that *P. madidus* moved further and faster, often crossing numerous hedgerows to move readily between crop types, and apparently favouring pea crops (*Pisum sativum*) over winter wheat (Holland *et al.* 2009). Both species, however, were less likely to move away from patches where activity-density was high than from those where it was relatively lower, suggesting that their overall spatial patterns were a presumably a consequence of the aggregation of a resource, which was probably prey (Holland *et al.* 2009).

When the spatial patterns of these beetles are sampled at smaller scales (i.e. with a sampling grid with 10-12 m intervals), aggregations of *P. melanarius* seem to be

less stable across the crop season than those of *P. madidus* (see Winder *et al.* 2005, Thomas *et al.* 2001, Thomas *et al.* 1998). In both cases, the species' spatiotemporal flux was a consequence of the beetles tracking aphid (Winder *et al.* 2001, 2005) and slug (Bohan *et al.* 2000) prey. An intensive mark-recapture study of *P. melanarius* (see Thomas *et al.* 1998) showed that periods of elevated activity-density followed increased rainfall. Whether this constituted a direct response to increases in humidity, or was a response to an increase in prey availability as a result of disturbance, was unclear (von Berg *et al.* 2008).

5.1.5. Innate and environmental influences on activity patterns

Most carabids, including *P. melanarius* and *P. madidus* are nocturnal (Greenslade 1963, Luff 1978, Chapman *et al.*1999). Circadian rhythms are innately mediated by an endogenous circadian clock (Berson *et al.* 2002). Some carabid species common to arable fields such as *Harpalus rufipes*, *Nebria brevicollis* and *Notiophilus bigutattus*, maintain the same circadian rhythms as adults and larvae, while cave-dwelling species, for example, which receive little or no light cues (*Zeitgeiber*) to entrain their circadian rhythms, also display diel activity patterns (Weber *et al.* 1994). Nevertheless, a varying degree of plasticity exists in the expression of circadian activity. Greenslade (1963), for example, compared the diel activity (i.e. levels of movement) of a number of carabids, and found that *P. madidus* expressed diurnal behaviour immediately after removal from grassland, but shifted to nocturnal activity within a week of exposure to higher light levels in an experimental setting. Conversely, *P. madidus* taken from woodland to the experimental setting remained consistently nocturnal. *P. melanarius*, meanwhile, were nocturnal in both natural habitats and under experimental conditions (Greenslade 1963).

Other environmental variables also interact with endogenous diel activity patterns. Chapman *et al.* (1999) used automatic time-sorting pitfall traps to measure the diel activity of *P. melanarius* between open (monocropped, weeded cabbages, *Brassica oleracea*) and dense (unweeded cabbage, intersown with clover, *Trifolium repens*) vegetation, and found that while the beetle's activity was nocturnal in both, it was more exclusively so in the open vegetation. In laboratory trials investigating the mechanisms underlying this behaviour, overall activity increased with humidity, while diurnal activity increased in treatments with lower light levels, irrespective of

humidity (Chapman *et al.* 1999). A positive relationship between activity, and therefore prey consumption, and temperature has also been demonstrated in many carabids, including *Pterostichus* spp. (see Ayre 2001, Mair & Port 2002).

5.2. Aims and objectives

The main aim of this chapter was to measure the predation rates of two common carabid generalist predators, *P. melanarius* and *P. madidus*, on a range of prey species including aphids, collembola and linyphiid spiders using PCR. IGP on linyphiids was assumed to be unidirectional (section 3.1.2). Additionally, the aim was to place these trophic interactions in a spatial context using SADIE analysis. It was also predicted that the predators' levels of activity-density would be positively related to the rates of predation on spiders, aphids and collembola.

Monte Carlo models were employed to test the hypothesis of density-dependent predation of each of the intraguild prey (spiders) along with aphids, to assess whether the addition of further prey species to the permutations would change the results of these tests. As generalists, the carabid beetles were expected to consume prey in proportion to its abundance. Where this was not the case, this chapter aimed to explain such deviations through parallel assessments of the spatial co-occurrence of the predators

This chapter also aimed to assess the spatial patterns of each predator and prey, by comparing each species' spatial distribution to the null hypothesis of randomness. Intraguild prey, that complete at least one reproductive cycle in the growing season, were expected to show a lagged response to their prey, while IG predators were expected to show aggregative response. Where first order prey suffered as a consequence of this aggregation from predators, this was expected to manifest as a local decline in population.

Therefore, any response to increased abundances in their prey can be considered to represent purely a behavioural response from these carabids. Responses to prey were tested by comparing the local prey cluster coefficients with subsequent changes in the local activity-density of *P. melanarius* and *P. madidus*.

5.3. Methods

5.3.1. Sample collection

Samples of invertebrates were collected in 2007 on a spatially-referenced grid of 16m intervals, from Long Hoos field (2.85 ha) in Rothamsted Research Institute, Harpenden, UK (lat: 51.812N, long: 0.373W). *Pterostichus melanarius* and *P. madidus* were sampled using large, dry pitfall traps containing clay balls to act as refugia. These were opened for a 12 h period overnight. The contents of the traps were emptied into plastic bags and frozen within 2 h of collection. Small arthropods (including linyphiids, aphids and collembola) were sampled in a similar manner (Vortis sampler) to those collected in Highfield in 2006 (section 3,3.1). Three sets of samples were taken while the wheat was at different stages: flowering (19^{th} - 22^{nd} June 2007: Zadoks scale 69-70), milky or mealy ripe ears (10^{th} - 13^{th} July 2007: Zadoks scale 73-85) and at harvest (30^{th} July - 3^{rd} August 2007: Zadoks scale 90-92).

5.3.2. Beetle gut dissection, DNA extraction and PCR screening

Beetle guts were dissected under sterile conditions and transferred to microcentrifuge tubes (section 2.2.1). DNA was extracted using a Qiagen kit according to the manufacturer's instructions (section 2.2.2). Each sample was subjected to a single PCR screening using the 'COM LINY' multiplex reaction (section 2.4.2, Appendix V) using fluorescent-labelled primers (Harper *et al.* 2005). The PCR products were subsequently separated by size on polyacrylamide gels on an ABI 3730 sequencer (Applied Biosystems, Carlsbad, CA, USA) using a 50 s injection to the capillary. The fragments were analysed in the same capillaries as samples from another study (King *et al.*, unpublished data). Therefore the standard 1 µL of PCR product for such an analysis was made up of 0.5 µL of the COM LINY multiplex and 0.5 µL of product from this different set of samples (King *et al.* unpublished data) along with Genescan™ 350 ROX (Applied Biosystems, Carlsbad, CA, USA) size standard and buffers. At least one water blank, PCR negative and positive controls including all potential peaks combined in a single well were included on each plate. Peaks denoting positive results were identified by viewing the electropherograms on Genemapper software (Applied Biosystems, Carlsbad, CA, USA).

Additionally, PCR products from the relevant feeding trials (i.e. *P. melanarius* fed *B. gracilis*, *Erigone* spp. and *T. tenuis*) (see Appendix VI and VII) were rerun at similar conditions to the field samples (with a two-fold dilution of the standard 1 μL of DNA in each capillary, as in the previous paragraph) in order to correctly to reflect the screening protocol (i.e. use of fragment analysis and fluorescent labelled primers). Samples were subjected to a minimum two-fold dilution to comensate for a similar dilution factor due to bein run with the products of another multiplex (the results of which will published elswhere), screened with the sequencer and interpreted using Genmapper v3.7 (Applied Biosystems, Foster City, CA, USA).

5.3.3. Statistical analysis

5.3.3.1. Feeding trials and Monte Carlo models of predator preference

Monte Carlo models were used to test whether two species of carabid, *P. melanarius* and *P. madidus*, consumed the spider species *B. gracilis*, *Erigone* spp. and *T. tenuis* and the aphid *S. avenae* at random (in proportion their abundance in the field). The models corrected for beetle feeding time, relative fragment decay during digestion and relative prey abundance. Parameterisation of the models for probable time of beetle feeding used data collected from timed pitfall trapping of *P. melanarius* in undersown cabbage (Chapman *et al.* 1999) and *P. madidus* in strawberry fields (Luff 1974). Differences in digestion rates of each spider DNA fragment in the guts of the beetles were incorporated using laboratory feeding trial data. The results of a single, diluted screening (see section 5.3.2) of each *P. melanarius*-linyphiid feeding trial were subjected to a binomial regression model (section 2.6) and the resultant intercepts and slopes (Appendix VI) used to parameterise the Monte Carlo randomisation models.

Two sets of simulations were run. The first set of models permuted just the spider predation data by both predators, using the relative abundances of adults of the three common linyphiids, *B. gracilis*, *Erigone* spp. and *T. tenuis*. A second set of simulations included also beetles that had consumed *S. avenae*, adding the numbers of generically counted (i.e. aphids of all species were lumped together) aphids into the values for prey abundances. The models followed the same process as that detailed in section 3.3.5.2. The simulations thus produced distributions of expected detection rates of each species' target DNA fragment. Two-tailed probablility tests

(Manly 1997a, b) were applied; where detection rates fell outside 95% confidence intervals of the predicted means, predation was considered significantly different from random.

5.3.3.2. Spatial analysis

Spatial co-occurrence was assessed using SADIE (section 4.3.3) in pairwise analyses for each carabid, each linyphiid, aphids and collembola. Additionally, the temporal flux of the carabids *P. melanrius* and *P. madidus* was assessed by comparing the co-occurrence of each species between each temporal sampling point.

5.3.3.3. Spatial analysis of predator aggregative response

As mobile predators with a lifespan greater than the experimental period, any local increase in *P. melanarius* or *P. madidus* is likely the result of aggregative response to prey (Bohan *et al.* 2000, Winder *et al.* 2001, section 5.1.2). Regression analysis was used to assess the local increase in beetle activity-density in the periods between sampling points. Thus, SADIE cluster coefficients (v_i or v_j) of prey species (collembola, aphids and each linyphiid species) were used as the explanatory variable in a regression in which the subsequent increase in activity-density of the carabid predators was the response. Response rates of predators (r_{PR}) were calculated as:

$$r_{PR} = N_{kPRt+1} - N_{kPRt}$$

where N represents the count (at point k), of predator species PR, on date t. The approach to these regression analyses differs from that of Winder *et al.* (2001) in that patch (v_i) and gap (v_j) cluster coefficients are regressed in a single slope. This seems appropriate given that the indices represent points along a single interval scale (Legendre & Legendre 1998).

5.3.3.4. Local response of prey to predator clustering

Conversely, the aggregation of predators at a sampling site may result in subsequent reductions in prey. Thus, the relationship between the clustering of predators at a site and the subsequent decline in prey was assessed using regression analysis. First, the logarithmic growth (or decline) (r_p) of first-order (i.e. phytophagous and detritivorous[10]) prey (aphids and collembola) were calculated using the formula:

$$r_p = \ln(n_{kpt+1}) - \ln(n_{kpt})$$

where n represents the count, at point k, of species p, on date t. These rates of decline were used as the response variable in separate regression analyses where the SADIE cluster coefficients (v_i or v_j) of each of the predators (carabids and linyphiids) were used as the explanatory variable.

The same analyses were also carried out for intraguild predation. But with rates of linyphiid (intraguild prey) growth (or decline) calculated as above (section 5.3.3.3).

5.3.3.5. Local response of prey to predation

A series of spatial analyses of the effects of predation on local prey populations were carried out. In each analysis, the response variable was the change in abundance of aphid, collembola or linyphiid prey (the last of which are intraguild prey). These changes were assessed between times t_1 and t_2 (i.e between June and July and between July and August sampling dates). The explanatory variable was the predation level by either *P. madidus* or *P. melanarius* at each sampling site in the field. This predation level was calculated in two ways. Firstly, the predation level was defined as the untransformed proportion of carabids testing positive for a particular prey species at a given sampling site. This was done for both *P. madidus* and *P. melanarius*. Secondly, for *P. melanarius* only (see below), the predation level was set as the weighted proportion of beetles testing positive for prey. Separate analyses were run for each predator with each prey at both time periods.

Weighted predation by *P. melanarius* was calculated by upward adjustment of predation rates of those prey species whose target fragments persisted for shorter

[10] But see Read *et al.* (2006).

periods than the others in the comparison. The fragment which was estimated to persist for the longest was that of the 272 bp 18S collembola DNA (Kuusk & Agustí 2007). The T_{50} of this fragment in *P. melanarius* was estimated from the meta-analysis of section 2.6 at 19.12 h (Fig. 2.1). Thus, predation indices for the other prey species in the analysis were adjusted according to the formula:

Correction factor for species A = 19.12 h / T_{50} species A.

5.4. Results

5.4.1. Beetle and spider populations

Activity-density varied between the two *Pterostichus* species. Levels of activity-density of *P. madidus* increased between June and July, then stayed consistent through to August. Numbers of *P. melanarius* pitfall-trapped, on the other hand, increased as the growing season progressed. Linyphiid species each peaked in June (Fig. 5.1).

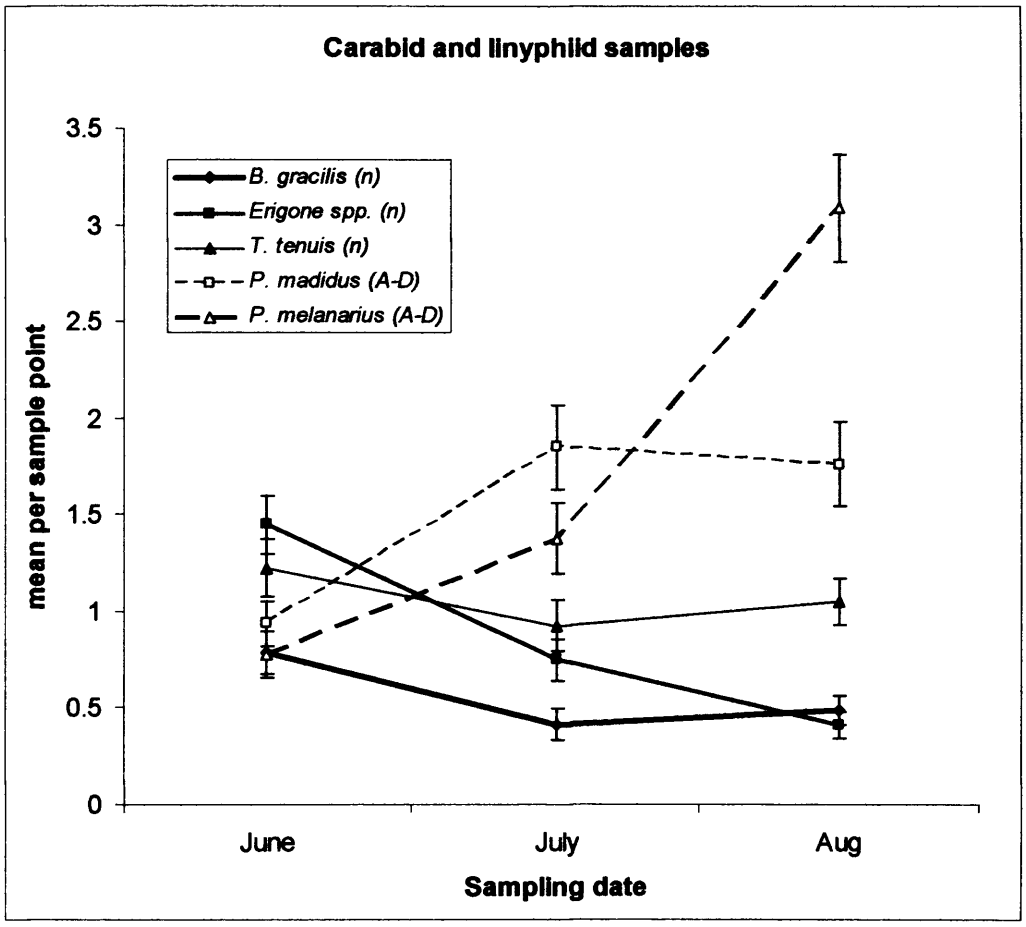


Fig. 5.1. Numbers of each carabid and linyphiid predator. Error bars represent SE. For the linyphiid, the figures represent absolute densities sampled at each point, while for the carabids, they are a measure of activity-density (pitfall trapping). Over the whole sampling season 419 *Pterostichus melanarius* and 364 *P. madidus* were trapped.

5.4.2. Predation rates by carabids on spiders, aphids and collembola

Absolute rates of predation (i.e. number of beetles testing positive for a prey fragment) are shown in Table 5.1. When predation by each carabid species was compared, it was found (after comparisons unadjusted by decay rates) that neither beetle species consumed more linyphiids than the other in any month (June: $\chi^2_1 =$

0.2976, P = 0.585, July: χ^2_1 = 2.248, P = 0.134, August: χ^2_1 = 0, P = 1.0). Their relative consumption of aphids did not differ in June either (χ^2_1 = 0, P = 1.0), but in July significantly lower proportion of *P. melanarius* consumed aphids than did *P. madidus* (χ^2_1 = 4.207, P = 0.040), while consumption by *P. madidus* was significantly higher than *P. melanarius* in August (χ^2_1 = 5.389, P = 0.020). More collembola were consumed by *P. madidus* than *P. melanarius* in all months (June: χ^2_1 = 4.146, P = 0.042, July: χ^2_1 = 19.367, P = < 0.0001, August: χ^2_1 = 15.663, P < 0.0001).

Month	Beetle	n (A-D)	*B. gracilis*	*Erigone* spp.	*T. tenuis*	Total linyphiids	*S. avenae*	Collembola
JUNE	*P. madidus*	75	1 (1.3)	3 (4.0)	8 (10.7)	12 (16.0)	10 (13.3)	20 (26.7)
	P. melanarius	62	0	4 (6.5)	3 (4.8)	7 (11.3)	9 (14.5)	7 (11.3)
JULY	*P. madidus*	148	1 (0.7)	5 (3.4)	13 (8.8)	19 (12.8)	19 (12.8)	43 (29.1)
	P. melanarius	110	0	5 (4.6)	2 (1.8)	7 (6.4)	5 (4.6)	7 (6.4)
AUG	*P. madidus*	141	1 (0.7)	1 (0.7)	9 (6.4)	11 (7.8)	29 (20.6)	43 (30.5)
	P. melanarius	247	2 (0.8)	10 (4.0)	7 (2.8)	19 (7.7)	28 (11.3)	33 (13.4)

Table 5.1. For each carabid species in each month, the number of beetles testing positve for each prey item is shown, with percentage of total beetles for the month in parentheses. n (A-D) represents activity-density (number trapped).

5.4.3. SADIE spatial analyses

Levels of *P. madidus* activity-density showed greater spatial stability over the course of the sampling period than *P. melanarius,* while the two carabid species were not significantly spatially associated during the season (Fig. 5.2). *P. madidus* remained significantly clustered into gaps and patches all year, while there was no relationship between June and July's cluster coefficients (X = 0.2347, P = 0.0278), but significantly stable (spatially concurrent) between July- August (X = 0.3714, P = 0.0002). *P. melanarius* on the other had showed a higher rate of spatiotemporal instability;, there was no association between the spatial patterns in July and August (X = -0.209, P = 0.5678). *Pterostichus madidus* and *P. melanarius* were not significantly associated with each other at any of the sampling occasions. *Pterostichus melanarius* were not significantly clustered during June or July, but were so in August. *Pterostichus madidus* meanwhile, were significantly clustered in every month, and comparisons between months showed that their distributions remained stable throughout the season. SADIE maps are shown in Fig. 5.2.

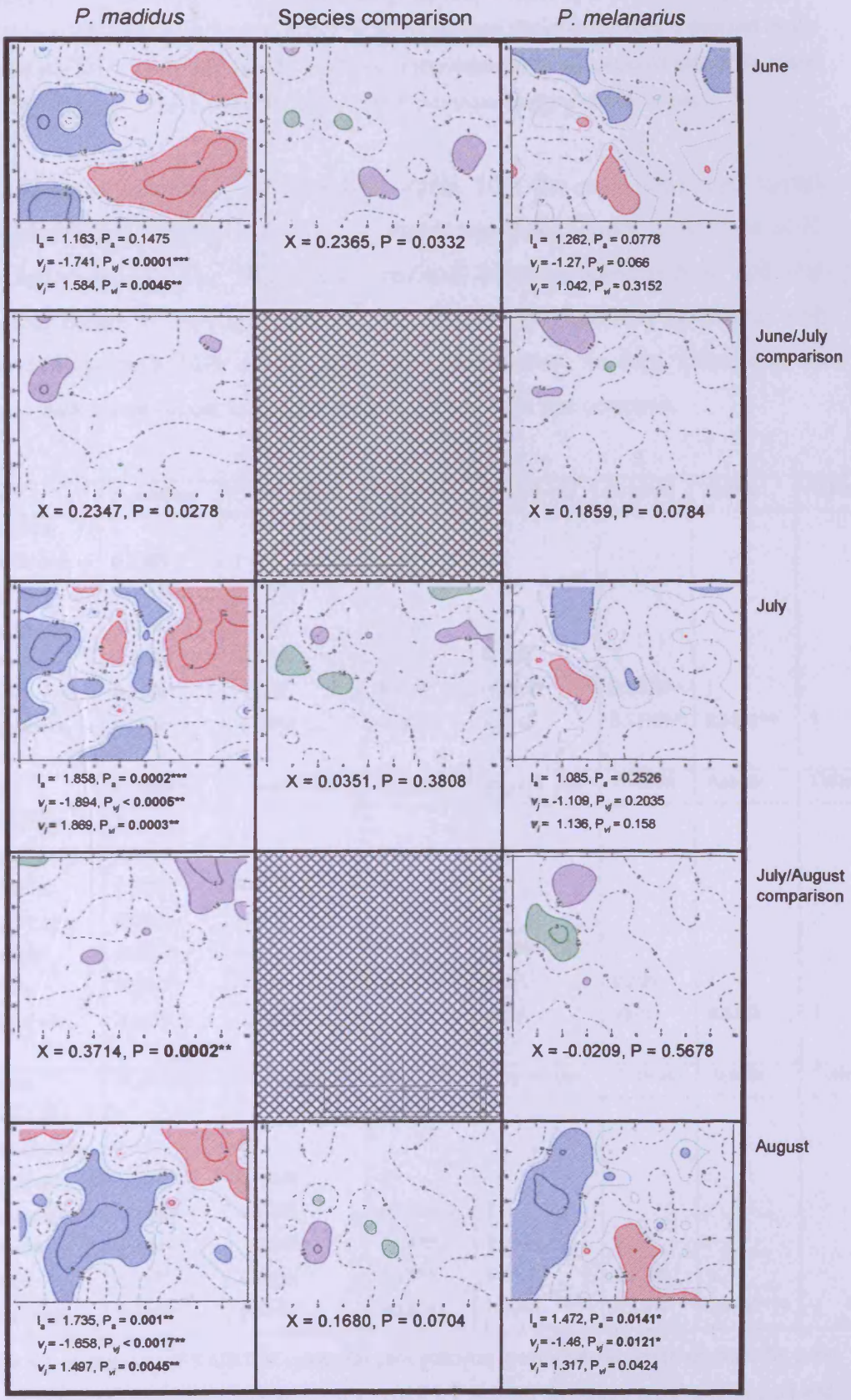

P. madidus
Species comparison
P. melanarius
June
June/July comparison
July
July/August comparison
August
I_a = 1.163, P_a = 0.1475
v_j = -1.741, P_{vj} < 0.0001***
v_i = 1.584, P_{vi} = 0.0045**
X = 0.2365, P = 0.0332
I_a = 1.262, P_a = 0.0778
v_j = -1.27, P_{vj} = 0.066
v_i = 1.042, P_{vi} = 0.3152
X = 0.2347, P = 0.0278
X = 0.1859, P = 0.0784
I_a = 1.858, P_a = 0.0002***
v_j = -1.894, P_{vj} < 0.0005**
v_i = 1.869, P_{vi} = 0.0003**
X = 0.0351, P = 0.3808
I_a = 1.085, P_a = 0.2526
v_j = -1.109, P_{vj} = 0.2035
v_i = 1.136, P_{vi} = 0.158
X = 0.3714, P = 0.0002**
X = -0.0209, P = 0.5678
I_a = 1.735, P_a = 0.001**
v_j = -1.658, P_{vj} < 0.0017**
v_i = 1.497, P_{vi} = 0.0045**
X = 0.1680, P = 0.0704
I_a = 1.472, P_a = 0.0141*
v_j = -1.46, P_{vj} = 0.0121*
v_i = 1.317, P_{vi} = 0.0424

Fig. 5.2. (previous page) SADIE red-blue plots depicting *Pterostichus melanarius* and *P. madidus* spatial patterns for each sampling occasion. Temporal flux is represented by the plum-green plots for the June-July and July-August comparisons while interspecific co-occurrence is depicted by the plum-green plots in the middle column. Each plot is accompanied by the associted indices (Ia, v_i and v_j for red-blue plots, SADIE X for plum-green) and their associated probability values.

Among the other spatial relationships (Table 5.2), the only significant spatial associations that persisted between successive sampling periods were those of *P. madidus* v. aphids. This relationship persisted between between June and July sampling points. *P. madidus* also was also significantly spatially associated with collembola in both June and August, but not, however, in July. Otherwise, no species were co-occurent with each other on more than one occasion.

June	*P. madidus*	*P. melanarius*	*B. gracilis*	*Erigone spp.*	*T. tenuis*	Aphids	Collembola
P. madidus	1						
P. melanarius	**0.2365**	1					
B. gracilis	0.0445	0.1964	1				
Erigone spp.	-0.1041	0.0967	-0.0127	1			
T. tenuis	0.1666	-0.0598	-0.1017	0.1481	1		
Aphids	**0.2273***	-0.111	0.0976	-0.0259	**0.2943***	1	
Collembola	**0.3321****	-0.0598	-0.0654	0.0312	**0.3772****	**0.3161****	1
July	*P. madidus*	*P. melanarius*	*B. gracilis*	*Erigone spp.*	*T. tenuis*	Aphids	Collembola
P. madidus	1						
P. melanarius	0.0351	1					
B. gracilis	0.0781	0.0513	1				
Erigone spp.	0.0361	0.142	0.1069	1			
T. tenuis	-0.0329	**0.212**	0.0606	-0.0956	1		
Aphids	**0.2767***	0.0515	0.0031	0.0451	**0.226**	1	
Collembola	0.1645	-0.0929	0.122	**0.218**	-0.013	**0.2282**	1
August	*P. madidus*	*P. melanarius*	*B. gracilis*	*Erigone spp.*	*T. tenuis*	Aphids	Collembola
P. madidus	1						
P. melanarius	0.168	1					
B. gracilis	**0.1985**	-0.0921	1				
Erigone spp.	-0.0269	-0.0243	-0.1501	1			
T. tenuis	-0.0284	-0.0808	0.1829	0.0044	1		
Aphids	0.1372	0.1496	0.1773	0.0322	**0.2249**	1	
Collembola	**0.2442***	**0.2849***	**0.1874**	0.0598	0.1908	**0.4827*****	1

Table 5.2. A sunmary of SADIE X values for each pairwise species interaction throughout the year. Where values are marginally significant (i.e. $0.025 < P < 0.05$) the values are shown in bold and where they are significant according to a two-tailed test, the number of asterisks denotes the level of significance.

5.4.4. Overall relationship between spatial co-occurrence and predation

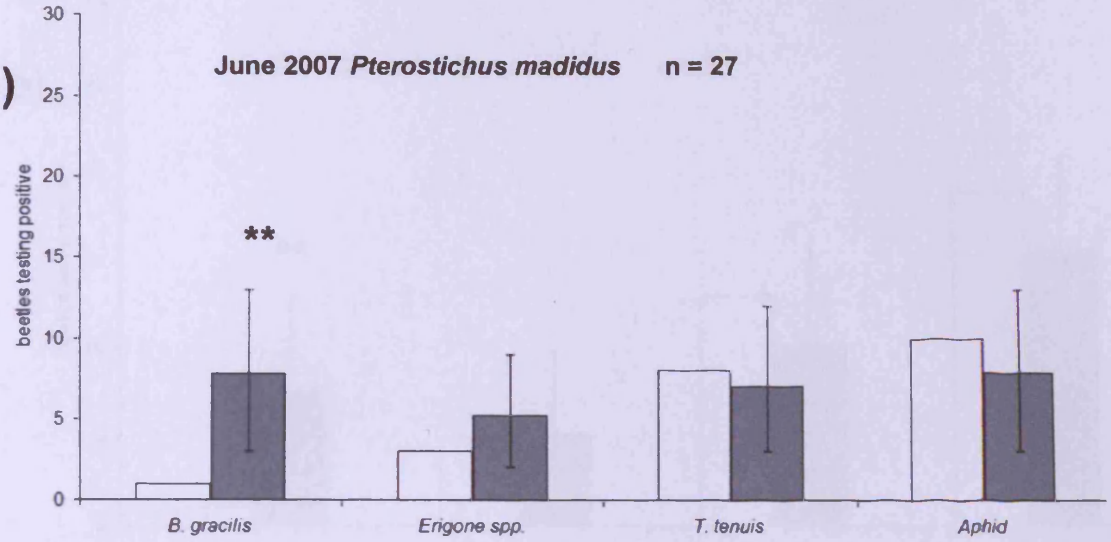
SADIE X values (section 5.4.3) of co-occurrence between each carabid and the five prey 'species', (*B. gracilis*, *Erigone* spp. *T. tenuis*, aphids and collembola) were used to predict average predation levels (i.e. across the whole field site). Separate analyses were undertaken for each month in a regression analysis.,Spatial proximity, as measured by SADIE X, did not predict predation rates either in June ($F_{1,8} = 2.591$, $r^2 = 0.245$, $P = 0.146$) or July ($F_{1,8} = 0.851$, $r^2 = 0.096$, $P = 0.383$). In August, however, SADIE X was significantly related to predation ($F_{1,8} = 10.62$, $r^2 = 0.570$, $P = 0.012$).

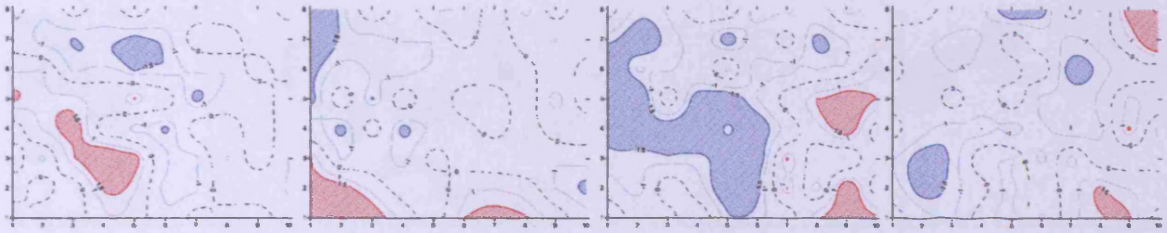
5.4.5. Monte Carlo prey choice models

Beetles which tested positive for prey were used as the basis for two distinct sets of Monte Carlo simulations. The first set dealt with those beetles testing positive for the three linyphiid species and the aphids, while a second resampled only the spider predators. These are presented alongside the blue-red SADIE maps for each of the predator and prey species and the plum-green co-occurrence maps depicting the spatial overlap between each prey with the relevant predator for each of the interacting species (Figure 5.3).

Fig. 5.3 (p123 - 128, parts a-e). At the top of each page, a barchart shows the results of the reasmpling of predation data from all beetles testing positive for either linyphiid or the aphid, *Sitobion avenae*. White bars show the observed rates of predation, while the grey bars show the expected predation levels, based on the probable feeding times of the beetles during their daily activity, corrected for the differences in detection times for each of the target amplicons and the relative numerical availability of each prey species. The error bars show 95% confidence intervals and the significance of any difference is as follos: *α = 5% **α = 1%, ***α = 0.01%. Below these are the SADIE red-blue plots (red is aggregation, blue diaggregation) depicting the spatial aggregation of each of the prey, along with aggregation indices I_a, v_j and v_i, and their associated probabilities (see section 4.3.3). Below these are the plum-green SADIE plots (where plum represents positive co-occurrence, green negative) depicting the spatial co-occurrence between each prey and the relevant predator, along with the indices of of overall co-occurrence SADIE X and its associated probability (section 4.3.3). The SADIE red-blue plot for the relevant predator is shown alongside the Monte Carlo results for predation without the inclusion of aphids (significance levels for these are the same as above). The number of beetles for which the predation data was resampled is displayed in each of the bar charts.

a)



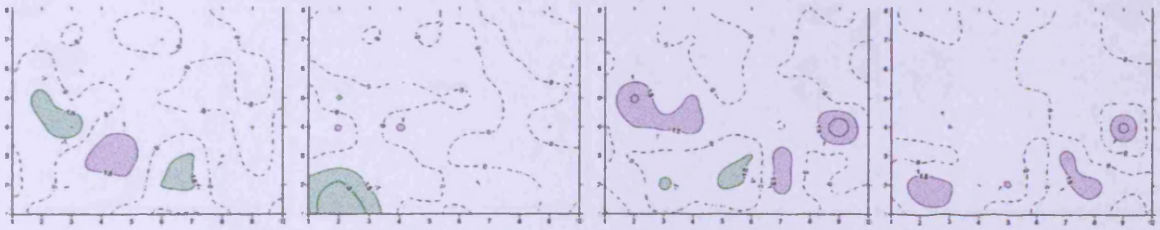


l_a= 1.015, P_a=0.3858
v_f= -1.013, P_{vf}=0.3762
v_f= 1.046, P_{vf}=0.2946

l_a= 1.163, P_a=0.1475
v_f= -1.065, P_{vf}=0.2765
v_f= 1.149, P_{vf}=0.1487

l_a= 1.387, P_a=**0.0238***
v_f= -1.379, P_{vf}=0.0265
v_f= 1.322, P_{vf}=0.0432

l_a= 1.002, P_a=0.4183
v_f= -1.046, P_{vf}=0.3281
v_f= 1.104, P_{vf}=0.2113

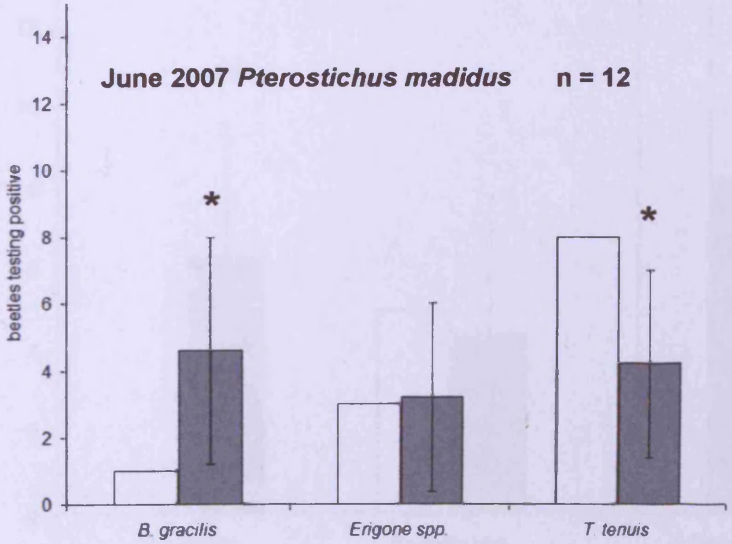


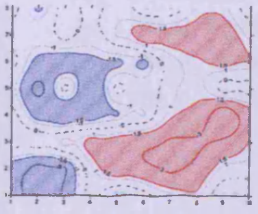
X = 0.0445, P = 0.3536

X = -0.1041, P = 0.8081

X = 0.1666, P = 0.0776

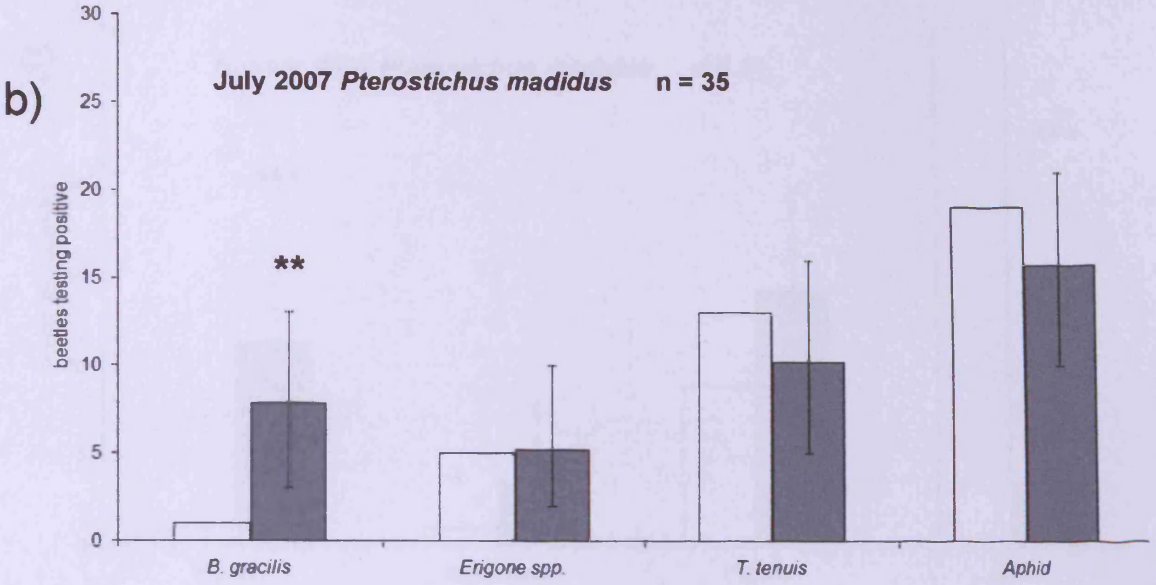
X = 0.2273, P = **0.0246***

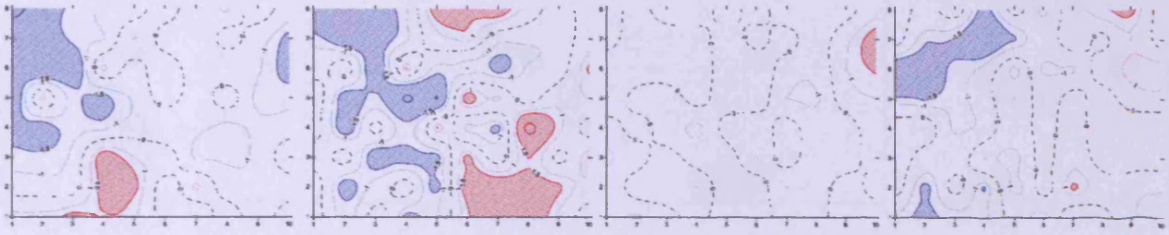




l_a= 1.742, P_a=**0.0005*****
v_f= -1.741, P_{vf} **<0.001****
v_f= 1.584, P_{vf}= **0.0045****

b)



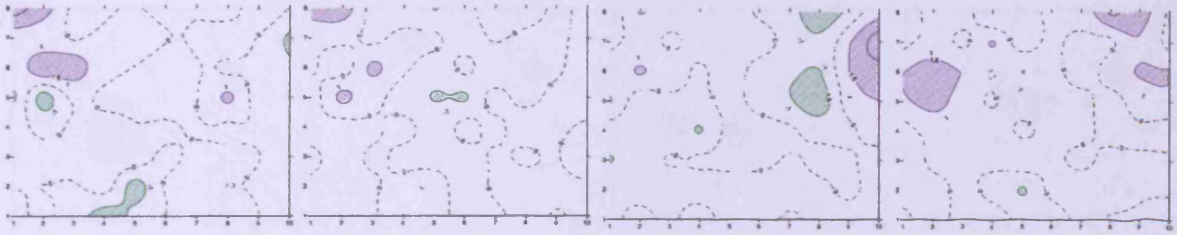


I_a= 1.186, P_a=0.1223
v_j= -1.193, P_{vj}=0.1168
v_i= 1.186, P_{vi}=0.1136

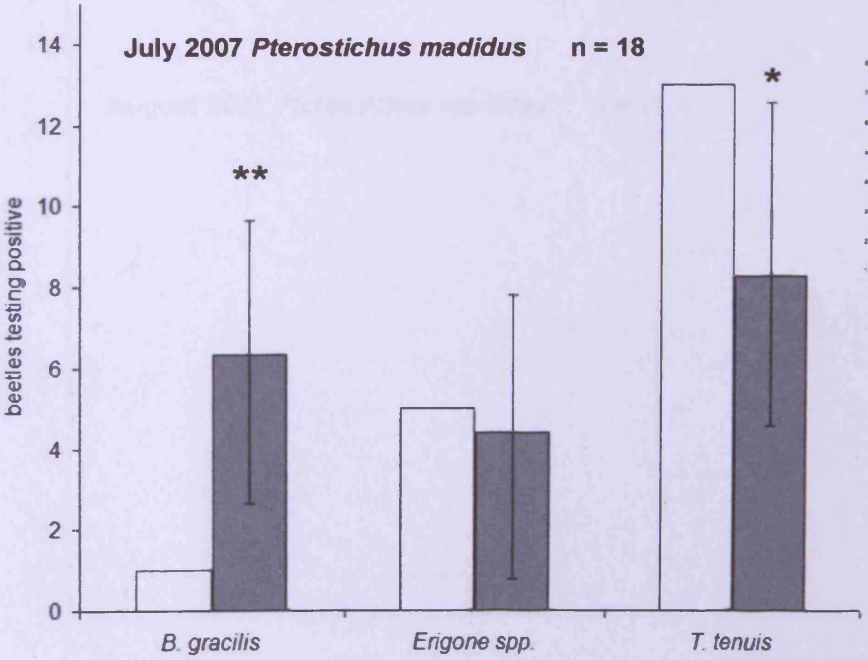
I_a= 1.152, P_a=**0.0077****
v_j= -1.517, P_{vj}=**0.0096***
v_i= 1.401, P_{vi}=**0.0194***

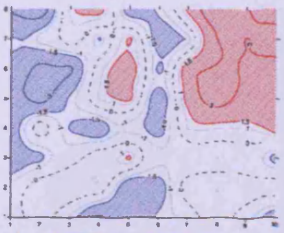
I_a= 1.736, P_a=**0.9928***
v_j= -0.737, P_{vj}=**0.9926***
v_i= 0.816, P_{vi}=0.9507

I_a= 1.119, P_a=0.2036
v_j= -1.122, P_{vj}=0.1946
v_i= 0.997, P_{vi}=0.4201

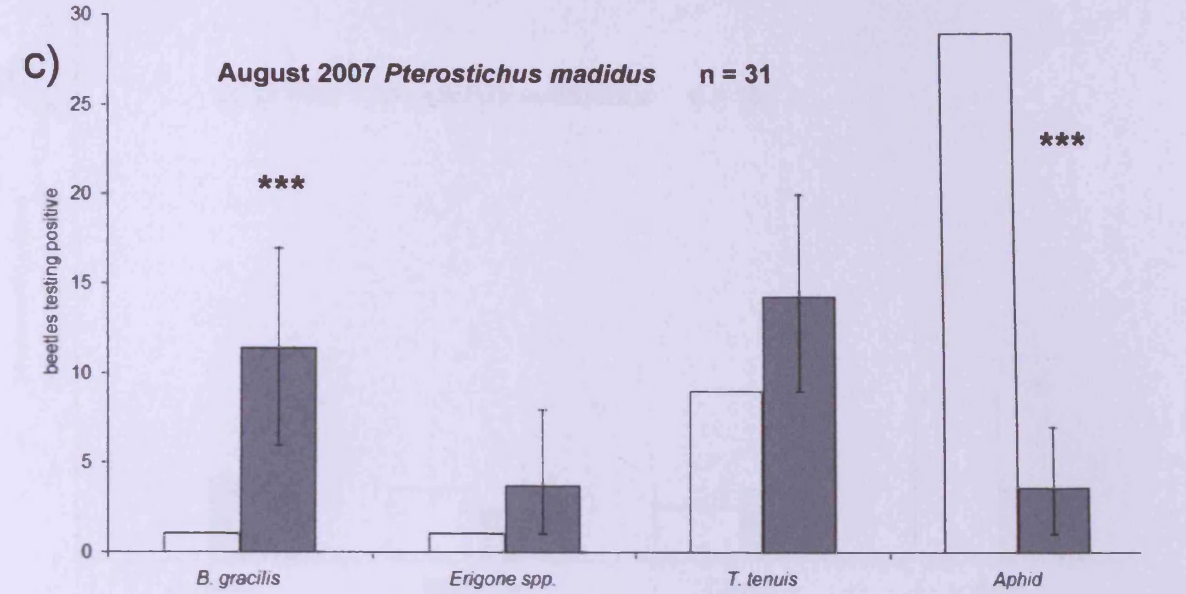


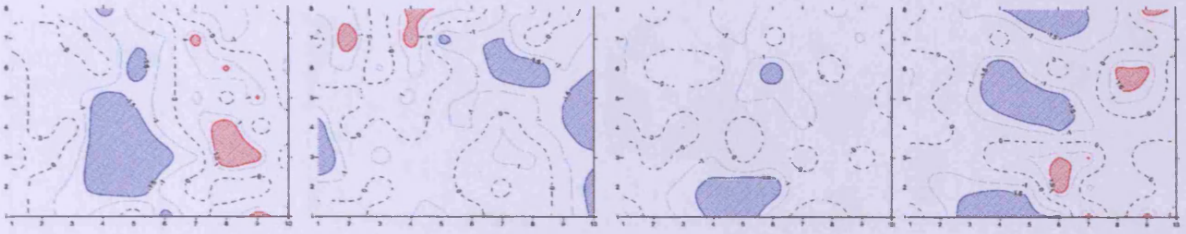
X = 0.0781, P = 0.2481
X = -0.0361, P = 0.3784
X = -0.0329, P = 0.6163
X = 0.2767, P = **0.0069***





I_a= 1.858, P_a=**0.0002*****
v_j= -1.894, P_{vj} **<0.0005****
v_i= 1.869, P_{vi}= **0.0003****



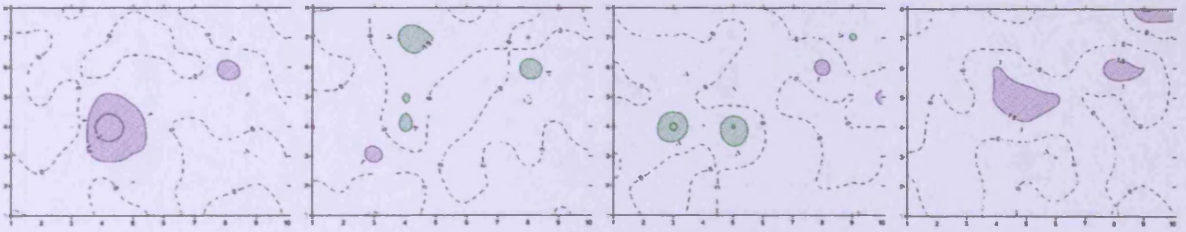


I_a= 1.151, P_a=0.1495
v_j= -1.145, P_{vj}=0.1564
v_i= 1.122, P_{vi}=0.1728

I_a= 1.124, P_a=0.1835
v_j= -1.127, P_{vj}=0.1735
v_i= 1.131, P_{vi}=0.1616

I_a= 0.847, P_a=0.8351
v_j= -0.891, P_{vj}=0.7407
v_i= 0.821, P_{vi}=0.9046

I_a= 1.147, P_a=0.1622
v_j= -1.136, P_{vj}=0.1788
v_i= 1.185, P_{vi}=0.1063

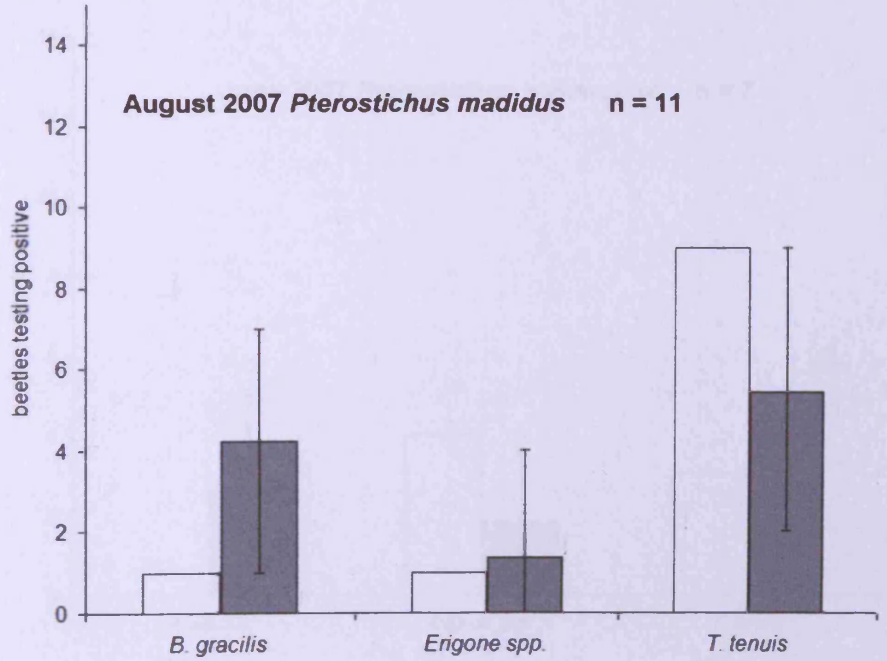


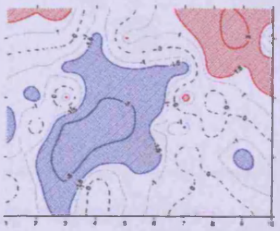
X = 0.1985, P = 0.0461*

X = -0.0269, P = 0.5863

X = -0.0284, P = 0.6005

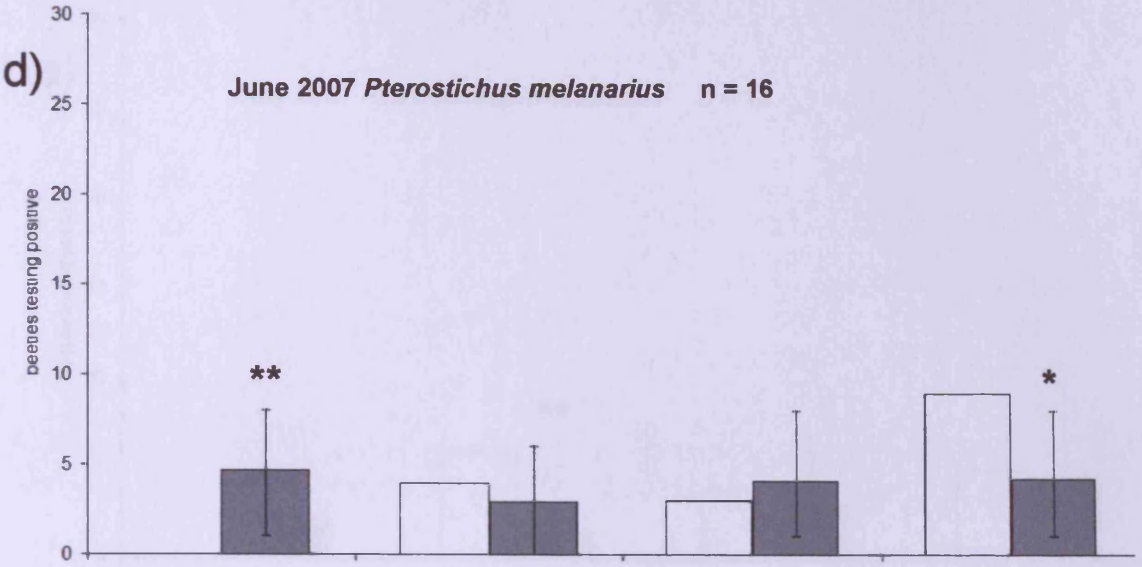
X = 0.1372, P = 0.1222

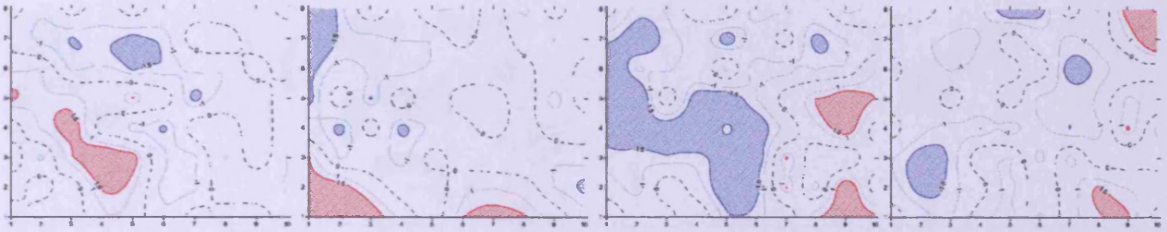




I_a= 1.735, P_a=**0.001****
v_j= -1.658, P_{vj} **<0.0017****
v_i= 1.497, P_{vi}= **0.0045****

d)



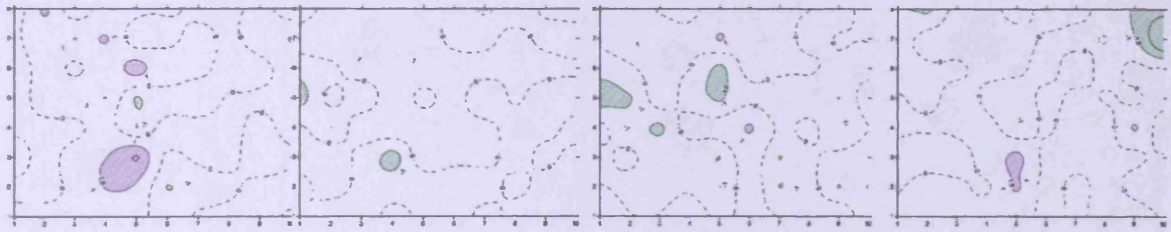


I_a= 1.015, P_a=0.3858
v_j= -1.013, P_{vj}=0.3762
v_i= 1.046, P_{vi}=0.2946

I_a= 1.163, P_a=0.1475
v_j= -1.065, P_{vj}=0.2765
v_i= 1.149, P_{vi}=0.1487

I_a= 1.387, P_a=**0.0238***
v_j= -1.379, P_{vj}=0.0265
v_i= 1.322, P_{vi}=0.0432

I_a= 1.002, P_a=0.4183
v_j= -1.046, P_{vj}=0.3281
v_i= 1.104, P_{vi}=0.2113

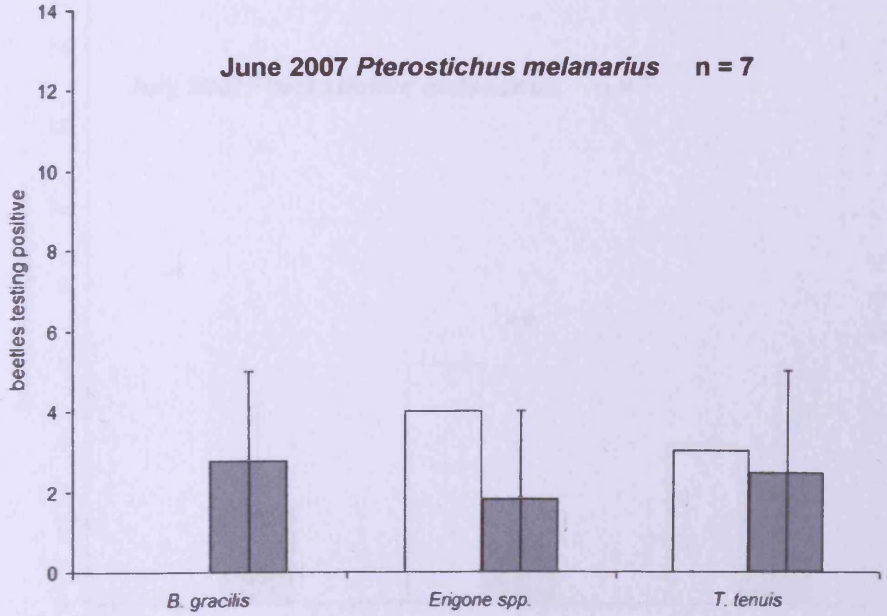


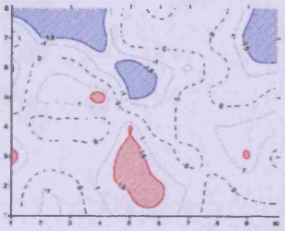
X = 0.1964, P = 0.0595

X = -0.0967, P = 0.1999

X = -0.0598, P = 0.6911

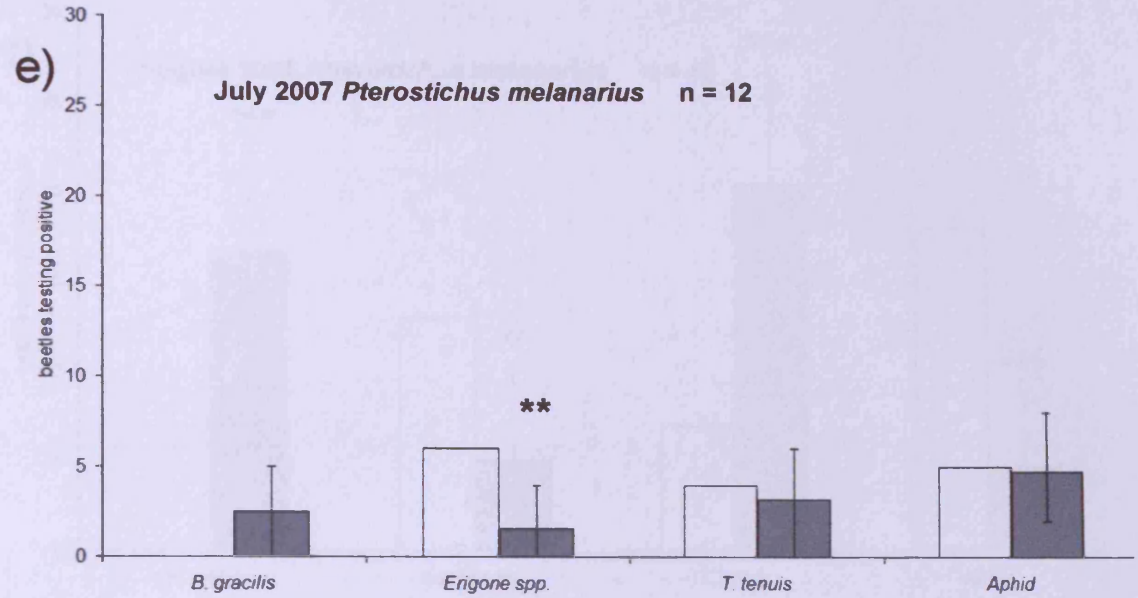
X = -0.0111, P = 0.8366

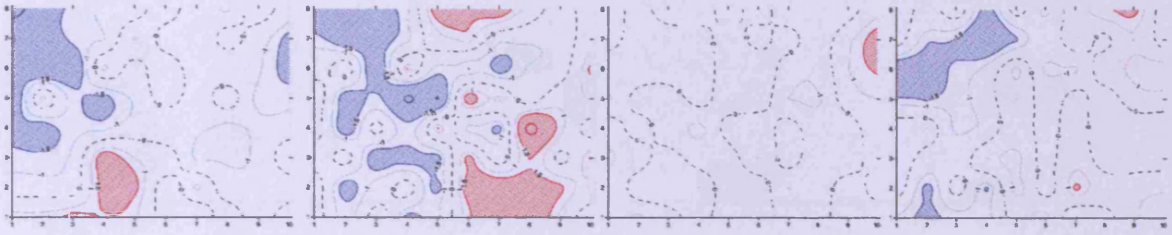




I_a= 1.262, P_a=0.0778
v_j= -1.27, P_{vj} <0.066
v_i= 1.042, P_{vi}= 3152

e)



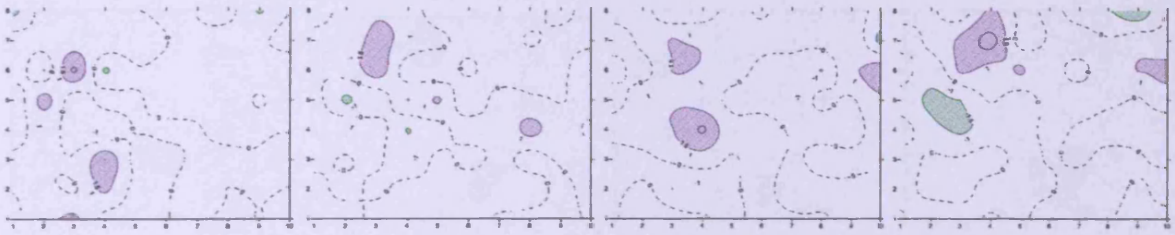


I_a= 1.186, P_a=0.1223
v_j= -1.193, P_{vj}=0.1168
v_i= 1.186, P_{vi}=0.1136

I_a= 1.152, P_a=**0.0077****
v_j= -1.517, P_{vj}=**0.0096***
v_i= 1.401, P_{vi}=**0.0194***

I_a= 1.736, P_a=**0.9928***
v_j= -0.737, P_{vj}=**0.9926***
v_i= 0.816, P_{vi}=0.9507

I_a= 1.119, P_a=0.2036
v_j= -1.122, P_{vj}=0.1946
v_i= 0.997, P_{vi}=0.4201

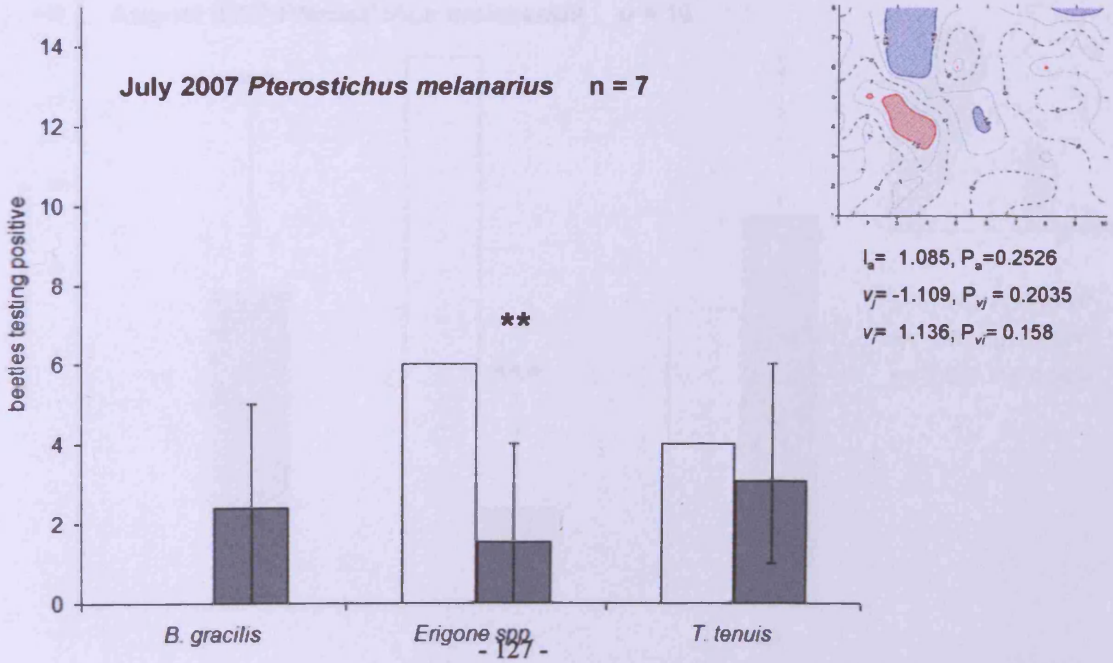


X = 0.0513, P = 0.3294

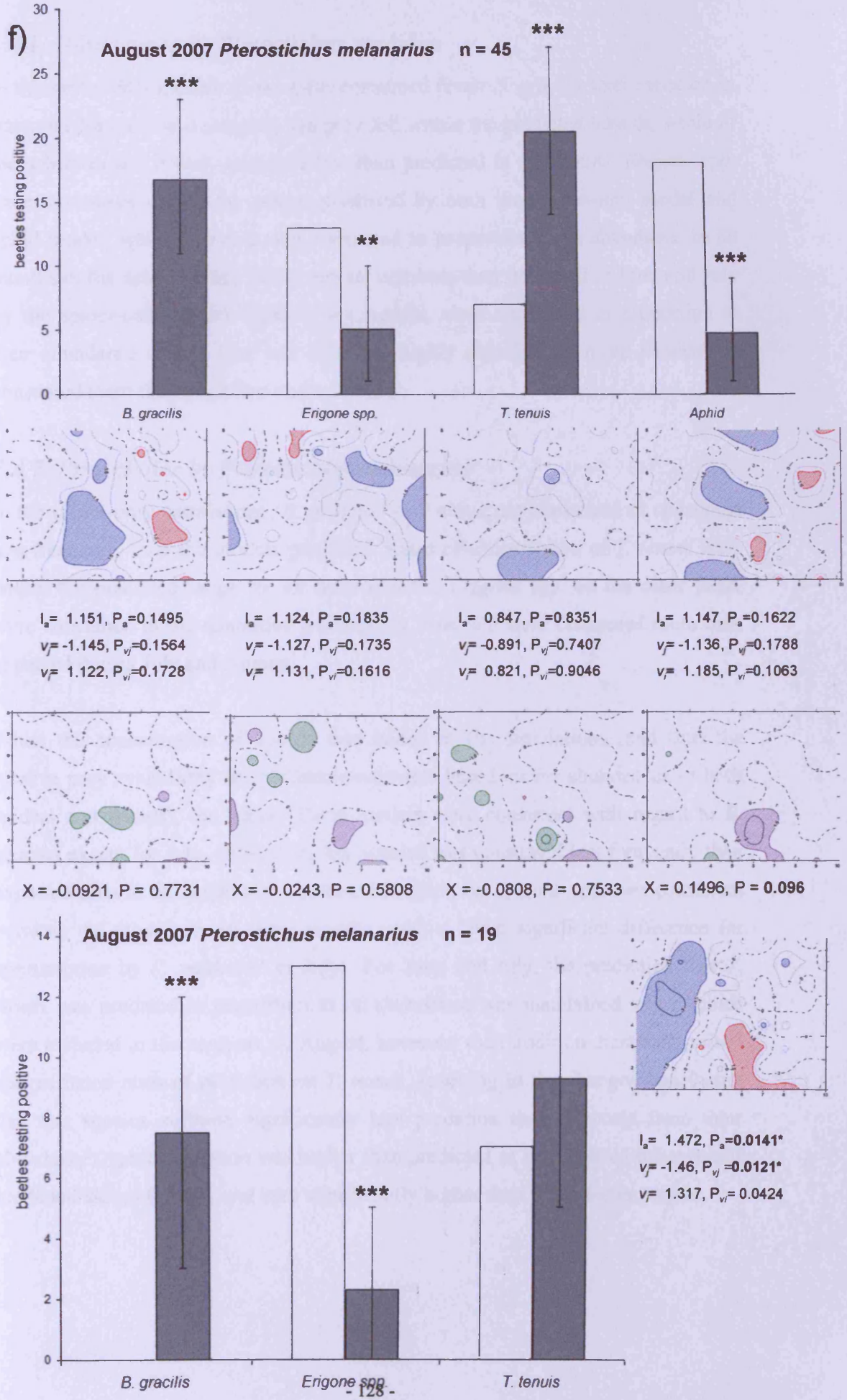
X = -0.0142, P = 0.1163

X = -0.0212, P = 0.0368*

X = 0.0515, P = 0.3381



I_a= 1.085, P_a=0.2526
v_j= -1.109, P_{vj} = 0.2035
v_i= 1.136, P_{vi}= 0.158


f)
August 2007 Pterostichus melanarius n = 45
beetles testing positive
***
**
***
***
B. gracilis
Erigone spp.
T. tenuis
Aphid
I_a= 1.151, P_a=0.1495
v_j= -1.145, P_{vj}=0.1564
v_i= 1.122, P_{vi}=0.1728
I_a= 1.124, P_a=0.1835
v_j= -1.127, P_{vj}=0.1735
v_i= 1.131, P_{vi}=0.1616
I_a= 0.847, P_a=0.8351
v_j= -0.891, P_{vj}=0.7407
v_i= 0.821, P_{vi}=0.9046
I_a= 1.147, P_a=0.1622
v_j= -1.136, P_{vj}=0.1788
v_i= 1.185, P_{vi}=0.1063
X = -0.0921, P = 0.7731
X = -0.0243, P = 0.5808
X = -0.0808, P = 0.7533
X = 0.1496, P = 0.096
August 2007 Pterostichus melanarius n = 19
beetles testing positive
***
***
B. gracilis
Erigone spp.
T. tenuis
I_a= 1.472, P_a=0.0141*
v_j= -1.46, P_{vj} =0.0121*
v_i= 1.317, P_{vi}= 0.0424

5.4.6. *Prey choice by* Pterostichus madidus

In the spider-only models *P. madidus* consumed fewer *B. gracilis* than expected in June and July, while in August, this prey fell within the predicted bounds, while in the aphid model, it was consumed less than predicted in all months. *Erigone* spp. were consumed within the ranges predicted by both the spider-only model and aphid model, while *T. tenuis* was consumed in proportion to its abundance in all months in the aphid model, but in greater numbers than predicted in June and July by the spider-only model. Aphids, meanwhile, were consumed in proportion to their abundance during June and July, but highly significantly more *P. madidus* consumed them than predicted during August.

5.4.7. *Prey choice by* Pterostichus melanarius

In the spider-only simulations, *B. gracilis* were either not consumed or consumed less frequently than the models predicted. Rates of consumption of *T. tenuis* were within the predicted range for all three months. *Erigone* spp. on the other hand, were consumed in the quantities predicted in June, but were consumed more than expected during July and August.

When the consumption of aphids was added to the simulations (and thus the relative prey availability for predation estimates based on the abundances of both spiders and aphids), the Monte Carlo models were consistent with regard to *B. gracilis* except for July. Otherwise, this species was consumed less frequently than expected than in the aphid-only models. Predation on *Erigone* spp. was consistent between the models in all three months (with a lower significant difference for consumption by *P. madidus'* in July). For June and July, the prediction that *T. tenuis* was predated in proportion to its abundance was maintained when aphids were included in the analysis. In August, however, their addition drastically raised the predicted rates of predation on *T. tenuis*, resulting in the changed conclusion that this species suffered significantly less predation than expected from their abundance. Aphid predation was higher than predicted in June, within the range of predicted values for July and very significantly higher than predicted in August.

5.4.8. Prey choice in relation to spatial co-occurrence

The Monte Carlo simulations including both spiders and aphids were assumed to be the most reliable indication of preferential predation (see discussion), and so only these findings are discussed here.

Co-occurrence between *B. gracilis* and the two carabid species varied considerably between sampling occasions, but levels of predation on this spider remained low. In all cases predation was either zero or below that predicted by the Monte Carlo models. The two occasions where predation on *Erigone* spp. was higher than expected corresponded to occasions where spatial co-occurrence, in terms of SADIE X, was highest (*P. melanarius* in July and August) (Figures 5.3, parts A-E). Similarly, where predation on *T. tenuis* was lower than expected according to the simulations, this corresponded to the lowest value of SADIE X (*P. melanarius* in August) (Figures 5.3, parts A-E). Such a relationship was, however, not apparent in the case of aphid predation.

5.4.9. Predator responses to prey clustering

Regressions were run to test how the growth of all predator species (carabids and spiders) responded to the spatial aggregation (SADIE cluster coefficients) of first-order (aphid and collembola) prey clustering. These showed no significant relationships. Full results are shown in Table 5.3

Predator	**Prey**	**Predator growth period**	**Slope**	$F_{1,78}$	r^2	**P**
B. gracilis ADULTS	Aphids - June	June-July	0.069	0.359	0.005	0.551
B. gracilis ADULTS	Aphids - July	July-Aug	0.106	1.053	0.013	0.308
B. gracilis ADULTS	Collembola - June	June-July	0.031	0.147	0.002	0.702
B. gracilis ADULTS	Collembola - July	July-Aug	-0.080	1.156	0.015	0.286
Erigone spp. ADULTS	Aphids - June	June-July	0.104	0.478	0.006	0.491
Erigone spp. ADULTS	Aphids - July	July-Aug	-0.065	0.315	0.004	0.576
Erigone spp. ADULTS	Collembola - June	June-July	0.070	0.438	0.006	0.511
Erigone spp. ADULTS	Collembola - July	July-Aug	-0.005	0.004	<0.001	0.950
T. tenuis ADULTS	Aphids - June	June-July	-0.119	0.514	0.007	0.476
T. tenuis ADULTS	Aphids - July	July-Aug	-0.223	1.899	0.024	0.172
T. tenuis ADULTS	Collembola - June	June-July	-0.228	3.910	0.048	0.052
T. tenuis ADULTS	Collembola - July	July-Aug	-0.125	1.152	0.015	0.287
P. madidus	Aphids - June	June-July	0.356	3.709	0.045	0.058
P. madidus	Aphids - July	July-Aug	-0.075	0.104	0.001	0.748
P. madidus	Collembola - June	June-July	-0.172	1.672	0.021	0.200
P. madidus	Collembola - July	July-Aug	0.176	1.11	0.014	0.29
P. melanarius	Aphids - June	June-July	0.054	0.097	0.001	0.757
P. melanarius	Aphids - July	July-Aug	-0.116	0.169	0.002	0.683
P. melanarius	Collembola - June	June-July	0.0070	0.003	<0.001	0.955
P. melanarius	Collembola - July	July-Aug	0.118	0.337	0.004	0.564

Table 5.3. Linear regressions to compare the local response of each predator (spider or beetle) to the local SADIE cluster coefficients of each of the first-order prey at the start of the period for which the population change was measured.

Similar tests of the response of carabids to the cluster coefficients of linyphiid intraguild prey produced no slopes significantly different form zero. Full results are shown in Table 5.4

Intraguild prey	Predator	Predator growth period	Slope	$F_{1,78}$	r^2	P
B. gracilis ADULTS - June	*P. madidus*	June-July	0.912	0.023	<0.001	0.880
B. gracilis ADULTS - July	*P. madidus*	July-Aug	-0.395	3.432	0.042	0.068
B. gracilis ADULTS - June	*P. melanarius*	June-July	0.224	1.594	0.020	0.210
B. gracilis ADULTS - July	*P. melanarius*	July-Aug	0.198	0.567	0.007	0.454
Erigone spp. ADULTS - June	*P. madidus*	June-July	0.317	3.300	0.041	0.073
Erigone spp. ADULTS - July	*P. madidus*	July-Aug	0.205	1.563	0.020	0.215
Erigone spp. ADULTS - June	*P. melanarius*	June-July	-0.062	0.145	0.020	0.705
Erigone spp. ADULTS - July	*P. melanarius*	July-Aug	0.321	2.637	0.033	0.108
T. tenuis ADULTS - June	*P. madidus*	June-July	0.153	1.164	0.014	0.284
T. tenuis ADULTS - July	*P. madidus*	July-Aug	-0.551	1.954	0.024	0.166
T. tenuis ADULTS - June	*P. melanarius*	June-July	0.127	0.680	0.009	0.412
T. tenuis ADULTS - July	*P. melanarius*	July-Aug	0.211	0.411	0.005	0.523

Table 5.4. Linear regressions to compare the local response of each intrguild predator to the local SADIE cluster coefficients of each of the linyphiid spiders (intraguild prey) at the start of the period for which the population change were measured.

5.4.10. Prey response to predator clustering

The relationship between responses (i.e. local population changes) of each of the first-order prey (aphids and collembola) to clustering of their putative predators was tested by regression analysis. A positive relationship between the cluster coefficients of *Erigone* spp. in June and the subsequent change in collembola populations was found ($F_{1,78}$ = 10.24, r^2 = 0.116, P = 0.002) i.e. association with *Erigone* spp. predicted a subsequent rise in collembola population. Additionally, the local clustering of *P. melanarius* in July was associated with a significant decline in collembola between July and August ($F_{1,78}$= 6.31, r^2 = 0.075, P = 0.014). Full results are presented in Table 5.5.

Predator	Prey	Prey growth period	Slope	$F_{1,78}$	Multiple r^2	P
B. gracilis ADULTS - June	Aphid	June-July	0.019	0.050	<0.001	0.827
B. gracilis ADULTS - July	Aphid	July-Aug	0.075	0.877	0.011	0.352
B. gracilis ADULTS - June	Collembola	June-July	0.176	1.713	0.021	0.194
B. gracilis ADULTS - July	Collembola	July-Aug	-0.257	3.679	0.045	0.059
Erigone spp. ADULTS - June	Aphid	June-July	-0.029	0.132	0.002	0.717
Erigone spp. ADULTS - July	Aphid	July-Aug	0.068	1.272	0.016	0.263
Erigone spp. ADULTS - June	Collembola	June-July	0.372	10.240	0.116	0.002**
Erigone spp. ADULTS - July	Collembola	July-Aug	0.052	0.251	0.003	0.617
T. tenuis ADULTS - June	Aphid	June-July	-0.004	0.003	<0.001	0.954
T. tenuis ADULTS - July	Aphid	July-Aug	-0.158	1.720	0.023	0.194
T. tenuis ADULTS - June	Collembola	June-July	0.0530	0.218	0.004	0.642
T. tenuis ADULTS - July	Collembola	July-Aug	-0.240	1.359	0.017	0.247
P. madidus	Aphid	June-July	0.054	1.144	0.015	0.288
P. madidus	Aphid	July-Aug	-0.083	3.364	0.041	0.070.
P. madidus	Collembola	June-July	-0.054	0.467	0.006	0.496
P. madidus	Collembola	July-Aug	-0.040	0.253	0.003	0.616
P. melanarius	Aphid	June-July	0.079	1.094	0.014	0.299
P. melanarius	Aphid	July-Aug	0.015	0.034	<0.001	0.855
P. melanarius	Collembola	June-July	-0.085	0.532	0.007	0.468
P. melanarius	Collembola	July-Aug	-0.340	6.307	0.075	0.014*

Table 5.5. Linear regresssions to compare the local response of each prey first-order prey species (i.e. aphids and collembola) to the local SADIE cluster coefficients of each of the predators at the start of the period for which the population change was measured.

Similar tests of the responses of intraguild prey populations to the clustering of carabids were all non-significant. Full details are shown in Table 5.6.

Predator	Prey	Prey growth period	Slope	$F_{1,78}$	r^2	P
P. madidus - June	*B. gracilis* ADULTS	June-July	-0.050	0.542	0.007	0.464
P. madidus - July	*B. gracilis* ADULTS	July-Aug	0.049	0.784	0.020	0.379
P. madidus - June	*Erigone* spp. ADULTS	June-July	0.093	1.099	0.014	0.298
P. madidus - July	*Erigone* spp. ADULTS	July-Aug	-0.019	0.094	0.001	0.760
P. madidus - June	*T. tenuis* ADULTS	June-July	-0.104	1.103	0.014	0.297
P. madidus - July	*T. tenuis* ADULTS	July-Aug	-0.107	1.466	0.018	0.230
P. melanarius - June	*B. gracilis* ADULTS	June-July	-0.086	0.717	0.009	0.400
P. melanarius - July	*B. gracilis* ADULTS	July-Aug	0.048	0.235	<0.001	0.630
P. melanarius - June	*Erigone* spp. ADULTS	June-July	-0.032	0.058	<0.001	0.811
P. melanarius - July	*Erigone* spp. ADULTS	July-Aug	-0.116	1.123	0.014	0.292
P. melanarius - June	*T. tenuis* ADULTS	June-July	-0.062	0.177	0.002	0.676
P. melanarius - July	*T. tenuis* ADULTS	July-Aug	-0.141	0.820	0.010	0.368

Table 5.6. Linear regression models to compare the local response of populations of each linyphiid spider species (i.e. intraguild prey) to the local SADIE cluster coefficients of each of the carabid predators at the start of the period for which the population change was measured.

5.4.11. Prey response to predation

Screening of the carabid predators with PCR afforded the opportunity to test directly whether prey declined as a consequence of local rates of predation. No significant relationships were found in the subsequent declines in any of the prey, whether or not the local predation rates were adjusted according to relative primer decay rates. Full details are shown in Table 5.7.

Predator	**Month**	**Prey**	**Prey growth period**	**Slope**	**F**	**d.f.**	**Multiple r^2**	**P**
P. madidus	June	*B. gracilis* ADULTS	June-July	-0.005	0.149	(1,44)	0.003	0.702
P. madidus	July	*B. gracilis* ADULTS	July-Aug	0.098	3.045	(1,50)	0.057	0.087
P. madidus	June	*Erigone* spp. ADULTS	June-July	0.013	0.331	(1,44)	0.007	0.568
P. melanarius	June	*Erigone* spp. ADULTS	June-July	-0.005	0.291	(1,39)	0.007	0.593
P. melanarius (w)	June	*Erigone* spp. ADULTS	June-July	-0.004	0.291	(1,39)	0.007	0.593
P. madidus	July	*Erigone* spp. ADULTS	July-Aug	0.012	0.353	(1,50)	0.007	0.555
P. melanarius	July	*Erigone* spp. ADULTS	July-Aug	-0.002	0.033	(1,44)	<0.001	0.856
P. melanarius (w)	July	*Erigone* spp. ADULTS	July-Aug	-0.001	0.033	(1,44)	<0.001	0.856
P. madidus	June	*T. tenuis* ADULTS	June-July	-0.009	0.947	(1,44)	0.021	0.336
P. melanarius	June	*T. tenuis* ADULTS	June-July	<0.001	<0.001	(1,39)	<0.001	0.995
P. melanarius (w)	June	*T. tenuis* ADULTS	June-July	<0.001	<0.001	(1,39)	<0.001	0.995
P. madidus	July	*T. tenuis* ADULTS	July-Aug	-0.001	0.002	(1,50)	<0.001	0.965
P. melanarius	July	*T. tenuis* ADULTS	July-Aug	0.018	0.381	(1,44)	0.009	0.540
P. melanarius (w)	July	*T. tenuis* ADULTS	July-Aug	0.014	0.381	(1,44)	0.009	0.540
P. madidus	June	Collembola	June_July	0.002	0.173	(1,44)	0.004	0.681
P. melanarius	June	Collembola	June_July	-0.001	0.015	(1,39)	<0.001	0.904
P. madidus	July	Collembola	July-Aug	-0.002	0.052	(1,50)	0.001	0.821
P. melanarius	July	Collembola	July-Aug	0.011	0.855	(1,44)	0.019	0.360
P. madidus	June	Aphids	June_July	-0.004	0.791	(1,44)	0.018	0.379
P. melanarius	June	Aphids	June_July	0.005	1.757	(1,39)	0.043	0.193
P. melanarius (w)	June	Aphids	June_July	0.002	1.757	(1,39)	0.043	0.193
P. madidus	July	Aphids	July-Aug	0.002	0.092	(1,50)	0.002	0.763
P. melanarius	July	Aphids	July-Aug	-0.013	3.133	(1,44)	0.066	0.084
P. melanarius (w)	July	Aphids	July-Aug	-0.004	3.133	(1,44)	0.067	0.083

Table 5.7. Linear regression models to compare the local response of populations of each of the intraguild prey and first-order prey to the local levels of predation by each of the carabid predators at the start of the period for which the population change was measured. (w) – weighted, adjusted for decay rates.

5.5. Discussion

5.5.1. Intraguild predation by carabids on linyphiids

Successful prevention of pest outbreaks relies on the maintenance of stable limit cycles of species in the crop. Communities in arable crops tend to be composed of species which display high rates of mobility, fecundity and growth because of the predictably destructive nature of annual cropping (Wissinger 1997). Intervention in the form of pesticide application usually serves to limit the growth of pests when they reach critical thresholds, but such interventions may have unpredictable results and are increasingly recognised as unsustainable (McLaughlin & Mineau 1995) because of their negative effects on biodiversity (Hole *et al.* 2005) and pollution (Cross & Edwards-Jones 2006, Stoate *et al.* 2001). Rather, a political imperative to harness the services of natural enemies as part of increased deployment of Integrated Pest Management (IPM) has recently emerged (section 1.2, Stoate *et al.* 2001).

Generalist predators are thought to forage in a density-dependent manner. Frequency-dependent selection of prey (Allen 1988, Abrams & Matsuda 2003) is thought to be a central mechanism by which limit cycling of populations will occur and in doing so lower the probability of pest outbreaks. Such prey switching would manifest as non-linear functional responses, and are predicted to stabilise systems with high levels of omnivory (Emmerson & Yearsley 2004). Elucidating functional responses is difficult as they depend on the population densities of numerous alternative prey (Abrams & Ginzburg 2000).

Here, data measuring predation by two carabid beetles on three different linyphiid spider species, aphids and collembola was compared to the levels of prey availability. These data was permuted 20, 000 times using Monte Carlo simulations to predict whether this predation was random, i.e. in proportion to relative prey availability. The two versions of the Monte Carlo simulations, with and without aphid predation (and aphid prey availability) included, gave broadly similar results. It should be noted that both models represent only a partial sample of the trophic niche of these generalist predators. Each species has, to some degree, consumed all

22 prey species that were screened for as part of a larger project[11] (King *et al.* 2010b, Harper et al. 2005, King *et al.* unpublished). The overall purpose of this wider[10] study, however, was not to try to establish the whole prey range of these predators, but to provide semi-quantitative measures of predation on the most important (i.e. numerous) prey groups. These Monte Carlo models can thus be thought of as representative of the partial trophic niches of the two species (Vandermeer 1972).

Similar studies of foraging carabids have shown predation to be density-dependent. King *et al.* (2010b) showed that *P. melanarius* showed no preference for epigieic over endogeic earthworms species and that defensive secretions of certain species did not, as hypothesised, reduce predation. This lack of prey choice between epigeic and endogeic worms was perhaps unsurprising given the beetles' abilities to track subterreanean prey by olfaction (Thomas *et al.* 2008). Hatteland *et al.* (in prep.) found, similarly, that the carabid *Carabus nemoralis* also exhibited no prey choice between species of pest slugs. Among their intraguild prey, however, while density-dependent foraging usually seems to be the case, the present study has shown some evidence of density independent foraging by *P. melanarius* and *P. madidus*.

Predictions of whether predation on each species was density-dependent were only inconsistent between the models in four out of 18 cases (two predators consuming three prey species measured on three occasions). The models included only those predators that had consumed any of the prey species. Hence, differences in the numbers of individual predators modelled in addition to the rates of prey availability will have influenced these outcomes; where more predation occurred, the numbers of predators permuted in the model would have risen, possibly providing smaller ranges of predicted predation rates. No formal comparison could be made between the two models, so it was therefore assumed that the inclusion of the aphids produced more reliable estimates, given their reflection of a larger part of the trophic niche of the beetles. Thus, further discussion will be restricted to these results. However, the models excluded collembola because they were found in such high abundance (Fig. 5.1). If tests of non-random predation had been run

[11] The BBSRC project: Dynamic Responses of predators to biodiversity in sustainable agriculture: Spatial and molecular analyses (BB/D001188/1)

with collembola included, these would have been trivial because of collembolas numerical dominance of all of the other prey species.

Where rates of predation were not density-dependent, differences in the diel overlap and spatial co-occurrence between predators' prey were hypothesied to affect these relationships. The spatial patterns of a species are a dynamic response to a number of factors (see section 4.1). Endogeneous locomotory activity patterns on the other hand, while exhibiting a degree of plasticity (section 5.1.4, 5.1.5), are in large part governed by innate internal clocks (Weber *et al.* 1994, Berson *et al.* 2002). There is also some evidence that the spiders' vertical spatial niches are also somewhat predetermined (Herberstein 1998, Harwood & Obrycki 2005).

During June and July, predation rates on linyphiids, aphids and collembola were not explained by their spatial proximity. However, in August, the relationships were significant. These anomolies may have been due to a number of factors. In the case of *B. gracilis,* predation was consistently lower than expected from density alone. This was expected, because this species places its webs furthest from the ground out of the three linyphiids under study (Sunderland *et al.* 1986). However, these spiders are often active on the ground, exhibiting high levels of temporal niche overlap (Pianka 1973) with the beetles. Pianka's niche overlap index was 0.769 between *B. gracilis* and *P. madidus* and 0.871 with *P. melanarius.* With the exception of *P. melanarius* in August, spatial co-occurrence between predators and *B. gracilis* was positive (i.e. SADIE X was > 0), but in no case significantly so.

Inferences regarding the spatial concurrence of the carabids and the strength of their trophic relationships with their prey rest on the assumption that carabids consumed their prey at the same location they were trapped. Mean daily dispersal rates, established using mark-recature studies show that *P. melanarius* can move 5.6 m per day, while in *P. madidus* this rises to 12 m, with the majority of males and females of both species moving less than 5 m per day (Holland *et al.* 2004, Thomas *et al.* 1998). This latter point implies that most of the beetles caught and screened in this experiment would have been likely to have actually resided and therefore consumed their prey nearer to where they were trapped than any other sampling point on the grid (sampling points were 16 m apart).

Erigone spp. spins its webs in small depressions in the ground, and thus may have been expected to suffer higher rates of predation than predicted by abundance alone because of this high vertical niche overlap with the two carabids. This was the case with *P. melanarius* in July and August, but predation by *P. madidus* was no higher than expected from relative prey densities. Where predation was density-independent, these differences could be explained by a combination of high spatial and temporal co-occurrence between *P. melanarius* and *Erigone* spp., SADIE X values were greatest when predation was non-random and the variation in values of Pianka's temporal niche overlap estimates were also conurrent with these patterns (0.912 with *P. melanarius* but only 0.390 with *P. madidus*) (Alderweireldt 1994a, Chapman et al. 1999).

Predation on *T. tenuis* was predicted by its abundance on all but one occasion, which was for *P. melanarius* at harvest (August). On this occasion, predation was significantly lower than expected. This corresponded to the occasion where spatial co-occurrence was lowest. The web heights of this species were intermediate between *B. gracilis* and *Erigone* spp., while their estimated diel overlap between these species was high (0.970 for *P. melanarius*, 0.830 for *P. madidus*).

5.5.2. Aphid predation by Pterostichus *spp.*

Pterostichus melanarius were not at any point significantly spatially aggregated with aphids, but nevertheless consumed them at a rate significantly higher than predicted by aphid abundance in June and August. Predation on aphids by *P. madidus* was even more inconsistent. On the two occasions where the species were significantly spatially aggregated, predation was density-dependent, while in August, when their levels of co-occurrence dropped, predation was significantly higher than expected. The sampling scale (which was geared to elucidating the spatial aggregations of carabids) may have failed to capture the spatial heterogeneity of aphids, which usually show aggregation at smaller scales (Winder *et al.* 1999, Fievet *et al.* 2007).

The search strategies of some carabids seem to benefit when their prey is more evenly distributed. This is well-documented for a close relative of *Pterostichus* spp., *Poecilus* (= *Pterostichus*) *cupreus*. Bommarco *et al.* (2007) demonstrated this using foraging models, while Griffiths *et al.* (2008) demonstrated similar patterns

in the field using SADIE analysis combined with mark-recapture and molecular gut analysis using ELISA. The continued decline of aphid populations once they had degenerated in to spatial randomness might have made them easier targets for other generalist predators such as *P. cupreus*, whose foraging strategy seems to benefit from widely dispersed populations of aphids (Bommarco *et al.* 2007).

Predation by carabids on aphids is often synergistic with the actions of other species, especially foliar foragers (Grez *et al.* 2007, Losey & Denno 1998, 1999) and suitable weather (von Berg *et al.* 2008), both of which cause aphids to drop to the ground whereupon they are easily preyed upon by ground-dwelling carabids.

5.5.3. Spatial analysis

Analysis of a number of spatial 'snapshots' throughout the season allows the elucidation of lagged responses of predators to prey (Winder *et al.* 2001, 2005, Bohan *et al.* 2000). Where the lifecycle of a predator takes place entirely in the crop and lasts for longer than the cropping season such patterns may be considered wholly behavioural responses. This is the case with both *P. menarius* and *P. madidus*. In more mobile species and/or with shorter lifecycles, any local increases may be from immigration from another field or other part of the crop or the result of a numerical response (i.e. an increase poulation through reproduction). In the latter case, one would more likely expect the rises in populations to consist of sub-adults, but given the capacity for immigration by linyphiids for their whole lives (ballooning), this cannot be ruled out as a source of higher numbers of juveniles.

These snapshot spatial analyses only showed strong coupling between populations of species in a limited number of cases. The significant negative relationship between *P. melanarius* clustering and collembola populations between July and August suggested that predation may have caused this decline, but when predation by *P. melanarius* was used as an explanatory variable for subsequent population change in collembola, the relationship was non-significant. Local reductions in collembola may have been due indirectly to predation on earthworms, whose casts attract collembola (Salmon & Ponge 2001). Additionally, local aggregations of *Erigone* spp. in June were associated with a subsequent rise in collembola numbers during June and July. Given that collembola constitute a substantial proportion of

the diet of *Erigone* spp., this was unexpected (Harwood *et al.* 2003, Alderweireldt 1994b) and probably implies that predation has little effect on population growth.

5.5.4. Implications for integrated pest management

Despite the presence of IGP, these findings suggest that no strong trophic couplings exist between any of the species examined in this chapter. Furthermore, there was only one occasion where IGP was greater than expected (given prey densities) and where simultaneously aphid predation was not. So IGP, from these results, could not be considered as diverting the predators from feeding on aphids. The ramifications of predation on linyphiids by carabids have been discussed in detail (section 3.5.3 - 3.5.4). The feeding habits of *Pterostichus* may not be entirely beneficial, however. Predation on linyphiid spiders (see also chapter 3) has the potential, for example, to reduce predation pressure on aphids, while consumption of diptera (King *et al.* unpublished data) may be beneficial if pests such as the Orange Wheat Blossom midge, *Sitodiplosis mosellana*, are taken or harmful if the flies are pollinators. In the case of predation on earthworms (King *et al.* 2010b, unpublished data) the feedback mechanisms may be even more complex. Earthworms act as soil 'engineers', influencing the availability of N to both plants and micro-organisms (Giannopoulos *et al.* 2010), which in turn regulates the rate at which N is fixed in the plant, which may subsequently determine rates of aphid reproduction (Ke & Scheu 2008).

6. General discussion

6.1. Synopsis

It is impossible to assess the efficacy of a natural enemy to arable crop pests outside its ecological context, elements of which include interactions between the landscape (Bianchi *et al.* 2006), the seasons (Wissinger *et al.* 1997), competitors (Harwood *et al.* 2005, Holland *et al.* 2009), predators (Polis *et al.* 1989), alternative prey (Abrams & Ginzburg *et al.* 2000, Agustí *et al.* 2003, Kuusk & Ekbom 2010), and the anthropogenic disturbance and inputs into the crop (Fadl *et al.* 1996, Holland *et al.* 2004, 2009). Lövei & Sunderland (1996) outline a process by which all of these elements may be incorporated, involving the following stages:

> " i) evaluating the dynamics of pest and predator co-occurrence,
> ii) establishing direct trophic links between these,
> iii) assessing the effects of a predator either by controlled feeding trials or field measurements of density of prey, predator and alternative prey,
> iv) synthesising this information in such a way that the effects of a predator on a prey may be predicted under various ecological or anthropogenic circumstances."

Carrying out these steps is challenging in the case of generalist predators, because their polyphagy makes prediction of their feeding habits difficult (Rosenheim & Corbett 2003). Here, the work undertaken in this thesis is summarised with a view to addressing Lövei & Sunderland's (1996) aims, and is hence presented in the same order.

6.2. Analysis of pest and predator dynamics

The dynamics of annual arable systems are characterised by their predictable ephemerality (Wissinger 1997). Conventional models of community assembly and population dynamics, which are based on equilibrium and underpinned by niche theory (Hutchinson 1957), are therefore inappropriate. Rather, community assembly follows stochastic rules and equilibrium (Tilman 2004, Hubbell 2005). Even in a homogenous crop, slight differences in soil condition may ramify

through the foodweb, and serve to create mosaics of different habitats through the actions of individual mobile elements (i.e. animals) (Hassell *et al.* 1991). Thus, to gain an insight into this dynamism in Northern European wheat fields, the work in this thesis largely comprised 'snapshots' of the distributions of aphids, collembola and linyphiid spiders sampled on three different occasions, and subjected to two different experimental settings (chapter 4, chapter 5).

The results of chapter 4 revealed that 'bottom-up' effects (those that originate from the level of the primary producer (Oksanen *et al.* 1981, see section 1.4), were prevalent in the experimental wheat field, as a consequence of the two different tillage regimes used. This is likely to have occurred because conventional tillage, which involves ploughing, provides a boost to wheat growth, leading to a higher crop yield (section 4.3, Rieger *et al.* 2008). Such effects are, however, likely to be unsustainable in the long term, as conventional tillage can introduce pulses of organic matter to the system that may increase the rate at which N is taken up by wheat. This can interact with the soil community to cause aphid outbreaks (Ke & Scheu 2008). Furthermore, ploughing may negatively affect carabid emergence (Fadl et al. 1996, Holland & Reynolds 2003).

While a negative relationship between aphid abundance and crop yield was expected due to the pest's deleterious effects on the crop (Entwistle & Dixon 1987), this study found that localities where yield was high were associated with elevated aphid abundance. This finding is likely to be the result of either differential settling by aphids on better quality host plants (Lushai *et al.* 1997), or the reproductive benefits to aphids associated with increased N in the phloem (Watt & Dixon 1981).

Aphid aggregation was measured in two experiments (section 4.3, 5.3). In the split plot tillage field experiment (chapter 4, 2006 data), aphids were aggregated at the start of the season, before degenerating into a random distribution (section 4.4, 5.4). In the other experimental setup, in which the tillage regime was uniform (chapter 5, 2007 data), the aphids did not significantly aggregate at any point (Section 5.4). Thus, these differences in the patterns of spatial aggregation of the aphids between the experimental setups were probably a conferred in a large part by the heterogeneity generated through the tillage treatments. The initial patterns of

aphid aggregation that were observed in the present study were similar to the findings of other studies (Winder *et al.* 1999, Fievet *et al.* 2007). However, as aphids were not measured at spatial scales smaller than a 16 m grid in this thesis, the results permit only speculation about whether the aphids maintain heterogeneity throughout the season. However, given the results of other studies, this is likely to be the case, with heterogeneity found at increasingly smaller scales as the season progresses (Winder *et al.* 1999, Fievet *et al.* 2007).

The higher local numerical responses which followed higher local clustering between linyphiid spiders and their pest (aphid) and non-pest (collembola) prey (section 4.3 – 4.4), suggested that early in the season, linyphiids played a valuable role in the biological control of aphids. This represents the first time the spatial co-occurrence between these protagonists has been measured. Polyphagous predators are expected to play their most valuable role in pest control early in the season, before more specialised natural enemies have a chance to reproduce (Winder *et al.* 1994, Settle *et al.* 1996, Chang & Kareiva 1999, Symondson *et al.* 2002a, Sigsgaard 2007). Östman *et al.* (2003) have shown that the actions of polyphagous predators serve to reduce the economic impact of the aphid *R. padi*, hence providing a valuable ecosystem service. Acting together, however, guilds of generalists and specialists are able to control pests to a greater degree than either guild alone (Schmidt *et al.* 2003). The reproduction of agrobiont spiders occurs during the crop season (Samu & Szinetár 2002), and may consist of more than one generation (Thorbek *et al.* 2003). Hence, linyphiid populations are dependent on prey availability in the field. In the split plot experimental system (section 4.4), it appeared that the linyphiid guild (*B. gracilis* and *T. tenuis* and *Erigone* spp.) effectively suppressed aphids. Predation rates by linyphiids on aphids are generally low, such that their pest control effects are largely attributable to their high web densities (Bilde & Toft 1997). Indeed, a large number of prey captured in the webs of linyphiids goes unconsumed (Sunderland 1999). These wasted kills are likely to end up in the detrivorous channel of the food web.

Web-owning *T. tenuis*, and therefore probably those *B. gracilis* able to defend a web-site, consume more aphids than non web-owning individuals (Harwood *et al.* 2004). The earlier arrival of a competitor for similar prey may prevent the late-coming individual from building its web at an optimal height (Herberstein 1998, Harwood &

Obrycki 2007). Thus, in many cases, such priority effects may affect the partitioning, in the horizontal plane, of niche space between *B. gracilis* and *T. tenuis*. Nevertheless, the two species occupy significantly different niches along the vertical spatial axis (Sunderland *et al.* 1986, Alderweireldt 1994b). Furthermore, this segregation has been found to increase as the season progressed (Sunderland *et al.* 1986) as a consequence of *B. gracilis* placing their webs at increasing heights from the soil surface. In the results presented here, the two species' horizontal spatial overlap increased as the season progressed. This was found in both experiments undertaken (section 3.3, section 5.3), and could represent a response to depleted prey and increased competition from other, later arriving invertebrates. It is possible, therefore, that the rising heights of *B. gracilis'* webs may be a reaction to increases in horizontal spatial niche overlap.

While providing efficient estimates of populations for all the species involved in this analysis (Topping & Sunderland 1998, Elliot *et al.* 2006), the vacuum sampling methods employed in the experiments (sections 2.7, 3.3, 4.3 and 5.3) make it impossible to determine where the linyphiines sampled (*B. gracilis* and *T. tenuis*) existed along the gradient from web-dependence to active hunting (Alderweireldt 1994b, Harwood *et al.* 2001, 2003). The sampling methods therefore also precluded establishing whether "trophic switches" (Rosenheim & Corbett 2003) were operating in the systems studied. The phenomenon of trophic switches was found to emerge from individual-based, spatially explicit models, used to investigate the role of predator function in determining trophic interactions. These models predicted trophic switches, as sit-and-wait predators were found to be more likely to consume mobile prey, while mobile predators were more likely to consume sedentary prey (Rosenheim & Corbett 2003).

Indeed, caution must also be exercised in any interpretation of the observed patterns of co-occurrence (Lang 2000). *Tenuiphantes tenuis*, for example, displays differential rates of airborne migration between sexes and life stages at different times of the season, while activity rates of males depend on whether or not they have mated (Topping & Sunderland 1998). Thus, abundance does not reflect activity, but neither does the amount of activity reflect the amount of time spent hunting for prey. A sampling regime combining activity-density sampling (with pitfall traps for example), absolute densities and immigration would be ideal (Lang 2000).

While a lagged numerical response to predator and prey aggregation may imply a trophic link, it is by no means certain that such trends are caused directly by energy flows between the species analysed (Winder *et al.* 2001). Nevertheless a number of studies have found such spatial patterns, and have combined them with predation measures to provide firmer, if still circumstantial evidence, that predation has taken place (Bohan *et al.* 2000, Winder *et al.* 2001, Winder *et al.* 2005). It is therefore possible, and indeed probable, that the results of the lagged responses by the linyphiids to the aphids and collembola (chapter 4, chapter 5) constituted an observation of predation by linyphiids of aphids, which could thus be considered an economically useful ecosystem service.

6.3. Direct identification of trophic links

While the scale of this investigation of IGP in agricultural systems is not unprecedented (Hengeveld 1980, Sunderland 1975), the resolution to which it has identified IG prey makes it unique. The only other study to date of IGP using PCR (involving predators as opposed to parasitoids) is that of Harwood *et al.* (2009), who monitored a soybean food web containing both the adult and juvenile Anthocorid bug, *Orius insidiosus*, and the alien coccinellid, *Harmonia axyridis*, each of which is a predator of the aphid, *Aphis glycines*. These authors discovered that *H. axyridis* showed low-level predation (2.5% testing positive) on juvenile *O. insidius*, but not on adults.

Much work examining trophic interactions in cereal crops to date has focused on the consumption of pest prey (e.g. Sunderland 1987, Winder *et al.* 1994, Harper *et al.* 2005, section 5.1.2), and more recently alternative non-pest prey (Agustí *et al.* 2003, Harwood *et al.* 2007, Kuusk & Ekbom 2010), while morphological gut content analysis can often provide resolution to at least Order level (Appendix X). The largest of these studies found over 20 species of carabids, including *P. madidus* and *P. melanarius*, to have consumed 'spider-coloured liquid food'[12] which was assumed to be mainly Lycosidae, while eighteen species of carabid, including *P. niger*, *P. oblopunctatus* and *P. versicolor*, contained trichomes which were identifiable as belonging to spiders (Hengeveld 1980). The work in this

[12] The only qualification was given for this, was that no other organisms sampled were a closer match to the colour of these remains.

thesis complements the latter study, by providing further information on the IG prey consumed by a small number of carabid beetle species to a high resolution.

The PCR technique employed in this thesis (chapter 2) established that high rates of IGP occur between carabid predators (IG predators) and common linyphiid and tetragnathiid spiders (IG prey), and that the strength of trophic links varied with sampling occasion, predator density and experimental system. *Tenuiphantes tenuis* and *Erigone* spp. were consumed, on occasion, at particularly high levels. While predation of *T. tenuis* and *Erigone* spp. were variable, rates of consumption of *B. gracilis* were consistently low in comparison with the other spiders. Such shifting patterns of predation were not unexpected from the polyphagous carabid predators. This is because they exist in a system in which spatial heterogeneity is similarly dynamic, and driven by organismal interactions (Hassell *et al.* 1991, Guy *et al.* 2008, Wallin & Ekbom 1994) (chapter 4, chapter 5).

Predation by *P. melanarius* and *P. madidus* on aphids and collembola was also monitored and compared in the uniform tillage plot experiment in section 5.3, in addition to the rates of predation experienced by the three linyphiid spider species. This direct observation of trophic interactions, in parallel with models of spatial co-occurrence, thus allowed a more robust test of whether predators responded to local abundances of prey, or vice versa. However, no significant interactions occurred, with inclusion of predation as a predictor of IG prey, pest or non-pest prey response (section 5.4.). Such results are encouraging from a biological control perspective, because they imply that none of the trophic links studied is sufficiently strong to cause destabilisation in the food web (Dunne *et al.* 2002, Bascompte *et al.* 2005, Gross *et al.* 2009). However, the evidence that linyphiids reduced aphid prey effectively, while neither carabid species did so, implies that these IG prey are superior to IG predators at exploiting this shared prey. This constitutes one of the fundamental requirements for coexistence between IG predator and IG prey, according to traditional, equilibrium models of predator coexistence (Polis & Holt 1997) (section 1.5).

A direct comparison was also made of the relationship between the spatial co-occurrence of predators with their prey (section 5.3) (see below). This relationship was only significant in August (section 5.4.6), at a time when fewer trophic

interactions conformed to the expectation that predation would be in relation to abundance (see below). Bell *et al.* (2010), using the same dataset, found that overall (i.e. all three months together), spatial co-occurrence predicted predation rates on a range of earthworms and the pest slug *D. reticulatum*. On one hand, this decoupling of predation rates from spatial co-occurrence makes prediction of trophic link strength difficult (section 5.5.3) and implies the potential for non-random interactions. On the other hand, the transience of their strengths may serve to stabilise predator-prey limit cycles within the season (Keeling *et al.* 2000, Tobin & Bjørnstad 2003, Goodwin *et al.* 2005).

6.4. Assessment of predation in relation to field abundance and alternative prey

A combination of predation data from PCR analyses (sections 3.4.3, 5.4.2) and simultaneously sampled prey populations were resampled in Monte Carlo models. The results suggested that the high rates of *T. tenuis* predation found in section 3.4.3 were probably a consequence of 'preference' by the predator, and that the low rates of *B. gracilis* predation were a consequence of avoidance. Predation was, however, mostly within the boundaries predicted by the Monte Carlo models (i.e density-dependent). In the uniform tillage plot experiment (chapter 5) there was no sampling occasion where more than one speceis of linyphiid was 'preferred' by either species of beetle. Furthermore, on the occasions when a linyphiid species was consumed preferentially, aphids were also 'preferred' (section 5.4.5). Again, from the biological control point of view, these results are encouraging, because it demonstrates that the beetles were not consuming linyphiids in preference to aphids. Inclusion of more predation data and abundances into the model would enable a larger range of the predators' trophic niches to be assessed (Vandermeer 1972).

6.5. Synthesis of data into a predictive framework

Schmitz (2007) examines the risks to prey species in the presence of single and multiple predators, by analysing a range of combinatorial studies (section 1.7), concluding that species' habitat domains and hunting mode were more important than species identity in predicting how they would interact. Further evidence for such predictions comes from studies of models (Rosenheim & Corbett 2003) These ideas have been addressed in relation to the hypotheses set out in chapters 3 and 5

of this thesis. It remains difficult when a large number of species employ flexible hunting strategies (Harwood *et al.* 2003), which serve to make both habitat domains and hunting mode difficult to determine (Rosenheim & Corbett 2003). In addition to spatial habitat domains, circadian activity has been raised as a potential predictor of levels of predator interaction.

The approach taken with the Monte Carlo models is similar to that of optimal foraging theory (OFT) (MacArthur & Pianka 1966, Charnov 1976). This is a framework in which a model is proposed, tested against expectations and subsequently refined. In this framework, a predator is hypothesised to optimise some nutritional quality of its resources, e.g. calorific content or a particular nutrient, given the constraints imposed by factors such as locating, subduing and handling the resource, whilst also avoiding danger from factors such as predation (Charnov 1976). Such an approach has proved successful in the case of herbivory and predation on sedentary prey, but less well where the prey are mobile (Sih & Christensen 2001). Thus, future refinements to the models could possibly include more detailed measures of spatial and temporal overlap e.g. incorporate endogenous diel activity of prey (e.g. Kruse *et al.* 2008), in addition to predators, and introduce a spatial element to the model to explicitly test the effects of scale.

6.6. Immigration, community assembly and synchronisation

Perhaps the most important factor that was not measured in this study was immigration and emigration of both pest species and IG prey. Linyphiids and winged stages of aphids are all capable of migrating distances larger than single fields (Llewellyn *et al.* 2003, Thomas *et al.* 2003, Bell *et al.* 2005). While the snapshots provided by SADIE give a measure of this process within the field, the levels of influx from the surrounding landscape will depend both on the weather and the composition of the surrounding landscape, i.e. the amount of non-crop natural habitat surrounding the farm such as grassland and forest (Schmidt *et al.* 2008). Stochastic models of community assembly (Tilman *et al.* 2004, Hubbell 2005) emphasise the importance of immigration in the assembly of ephemeral communities. Diversity at a site is determined more by the competitive interactions between established species and new recruits, than by competition between individuals already resident, essentially because of priority effects (Harwood & Obrycki 2005, Herberstein 1998). In such situations, the more propagules of a

species arriving, the more likely a species will establish. The close phenological synchronisation between crop disturbances and spiders common to arable crops (Samu & Szinetár 2002) may possibly act to ensure generalist predators become satiated with intraguild prey, thus maximising an individuals chances of survival.

The first stage of adopting a more predictive approach (would be the calculation of functional responses to certain prey, from which one can predict the numerical response. The functional response represents the number of prey killed by a predator over a given unit time (Holling 1959a, b). This, along with the subsequent numerical responses to the consumption of a prey, governs population regulation in predator-prey systems and is an invaluable tool in predicting outcomes (McCann *et al.* 1998, Daugherty *et al.* 2007, Holt & Huxel 2007). Functional responses can be divided into three types. A type I response in where the amount of prey consumed increases linearly in relation to its density, while in a type II this relationship is asymptotic and type III sigmoidal. As prey become more numerous and hence opportunities to attack them increase, the predator spends more time handling the prey than searching for or subduing them, such that the rate of prey consumption rises to an asymptote (Holling 1959a, b). Type II and type III responses share this property. They differ in that the curve of a type III response has an inflection point i.e. accelerates, then asymptotes (Holling 1959a, 1959b). This inflection point is thought to represent the density at which a predator will switch to an alternative, more numerous (or more apparent) prey (Holling 1959a, b).

Assessing such responses in nature is somewhat intractable, especially in the presence of multiple alternative prey (Abrams & Ginzburg 2000). There is little agreement, for example on whether predators' responses to prey depend more on the ratio between predator and prey or simply on the density of prey (Abrams & Ginzburg 2000), irrespective of alternative prey. Joly & Patterson (2003) propose a solution of dealing with data where predators have a choice of prey. This involves selection indices (Manly *et al.* 1972) to elucidate the shape (type II or type III) of the response based on direct observations of predation. However, this method relies simply on a choice between two prey and knowledge of which is primary and which the secondary prey. Futhermore, the application of these models reveals that the shape of the predator function is indeterminate, depending on the

interaction of handling time and the ratios of primary and alternative prey (Joly & Patterson 2003).

6.7. Food webs perspective

Lawton (1999) argues that any study at intermediate organisational scales will be subject to too many contingencies to observe any generalisable 'laws', from which one can make predictions about outcomes. Therefore, questions about how species richness and abundance affect ecosystem services such as pest control, and thus how they are affected by human disturbance, benefit from being approached at the level of whole food webs (Bascompte 2009, Ings *et al.* 2009, Macfadyen *et al.* 2009). Stable networks are often characterised by a non-random distributions of links (McCann *et al.* 1998, Dunne *et al.* 2002) among nodes and non-normal distributions of interaction strengths among these links (Bascompte *et al.* 2005, Wootton & Emmerson 2005), a pattern evident among the trophic links shown in this thesis, along with in other studies of trophic interactions in winter wheat (Agustí *et al.* 2003, Harwood *et al.* 2005) and alternative agricultural systems. Insights into the mechanics of species interactions can be gained by attempting to predict the structure of food webs from characteristics of species such as body size and its influence on optimal foraging decisions (Beckerman *et al.* 2006, Petchey *et al.* 2008), spatial co-occurrence (Bell *et al.* 2010) or in single- (Williams & Martinez *et al.* 2000) or multi-dimensional niche models (Allesina *et al.* 2008), which is a process which relies on precise, high-resolution phenomenological food web data such as that presented here.

6.8. Future directions

Future observations of the dynamics of similar species would benefit from smaller temporal and spatial scales of sampling (Winder *et al.* 1999, Bohan *et al.* 2000). The spatial scale at which an organism views its environment (its 'grain') changes with its size and with the topographical complexity (Wiens *et al.* 1995). The minimum recommended number of points for a grid for SADIE analysis is 6 x 6 (Perry *et al.* 1999). Therefore such changes in resolution could be achieved with the same amount of effort in processing and screening samples. Establishing the dynamics of relatively fast-breeding species such as aphids, collembola and linyphiids requires higher temporal resolution also, especially given the bivoltine

life cycles of many linyphiids common in annual crops (Thorbek *et al.* 2003). More sensitive collection methods are required to enable the molecular screening of smaller arthropods without the risk of, or having quantified the extent of, false positive links (Remén *et al.* 2010). This is particularly important, where the hypothesis is the existence of large numbers of weak trophic links in a food web.

This thesis presents a series of methods designed to establish reasonable estimates of trophic interaction strength (section 2.6) in a pragmatic manner and with minimal effort. However, when there is potentially a multitude of trophic interactions, a more informative approach might be to simply glean a knowledge of the binary links that make up reticulate food webs (Gross *et al.* 2009, Kones *et al.* 2009). Such multiple trophic interactions were almost certainly operating, and therefore influencing, the results presented herein, especially when considered in the context of the wider study of which this thesis forms a part. The recent and continuing development of pyrosequencing represents an efficient means of establishing binary trophic interactions in a semi-quantitative way (Valentini *et al.* 2008), and could therefore be usefully applied in any continuation of this study. The general primers reported in section 2.4.2 could be used with pyrosequencing to reveal trophic links between any given predator and linyphiid spiders, for example. Where potentially important trophic links are revealed, quantitative PCR (qPCR) could then be employed provide a robust estimation of an individual's rate of predation (MacMillan *et al.* 2006, Passmore *et al.* 2006, Troedsson *et al.* 2009).

Another possible way in which the work of this thesis could be extended is through the incorporation of local prey availability and prey diel activity in the sort of Monte Carlo models utilised (sections 3.3.5, 5.4.5). This could contribute to the predicting predation on mobile prey, the absence of which is recognised as a short-coming in the optimal foraging paradigm (Sih & Christensen 2001).

Bibliography

Abbas, K.A., Lasekan, O. & Khalil, S.K. (2010) Recent advances, advantages and limitations of genetically modified foods: A review. *Journal of Food, Agriculture and Environment*, **8**, 232-236.

Abrams, P.A. & Fung, S.R. (2010) Prey persistence and abundance in systems with intraguild predation and type-2 functional responses. *Journal of Theoretical Biology*, **264**, 1033 - 1042.

Abrams, P.A. & Ginzburg, L.R. (2000) The nature of predation: prey dependent, ratio dependent or neither? *Trends in Ecology & Evolution*, **15**, 337-341.

Abrams, P.A. & Matsuda, H. (2003) Population dynamical consequences of reduced predator switching at low total prey densities. *Population Ecology*, **45**, 175-185.

Admassu, B., Juen, A. & Traugott, M. (2006) Earthworm primers for DNA-based gut content analysis and their cross-reactivity in a multi-species system. *Soil Biology & Biochemistry*, **38**, 1308-1315.

Agustí, N., Shayler, S., Harwood, J., Vaughan, I., Sunderland, K. & Symondson, W. (2003) Collembola as alternative prey sustaining spiders in arable ecosystems: prey detection within predators using molecular markers. *Molecular Ecology*, **12**, 3467-3475.

Alderweireldt, M. (1994a) Day-night activity rhythms of spiders occuring in crop-rotated fields. *European Journal of Soil Biology*, **30**, 55-61.

Alderweireldt, M. (1994b) Prey selection and prey capture strategies of linyphiid spiders in high-input agricultural fields. *Bulletin of the British Arachnological Society*, **9**, 300-308.

Alderweireldt, M. (1989) Seasonal migration and the importance of edge zones for the survival of *Bathyphantes gracilis* (Blackwall, 1841) (Araneae; Linyphiidae) on high input crop fileds. *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent*, **54**, 835.

Alderweireldt, M. & de Keer, R. (1990) Field and laboratory observations on the life cycle of *Pachygnatha degeeri* Sundevall, 1830 and *Pachygnatha clercki* Sundevall, 1823 (Araneae, Tetragnathidae). *Acta Zoologica Fennica* **190**, 35-40.

Alhmedi, A., Haubruge, E. & Francis, F. (2010) Intraguild interactions and aphid predators: biological efficiency of *Harmonia axyridis* and *Episyrphus balteatus*. *Journal of Applied Entomology*, **134**, 34-44.

Allen, J. (1988) Frequency-dependent selection by predators. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **319**, 485-503.

Allesina, S., Alonso, D. & Pascual, M. (2008) A General Model for Food Web Structure. *Science*, **320**, 658-661.

Allsopp, M.H., de Lange, W.J. & Veldtman, R. (2008) Valuing Insect Pollination Services with Cost of Replacement. *PLoS ONE*, **3**, e3128.

Alvarez, T., Frampton, G.K. & Goulson, D. (2000) The role of hedgerows in the recolonisation of arable fields by epigeal Collembola. *Pedobiologia*, **44**, 516-526.

Alvarez, T., Frampton, G. & Goulson, D. (2001) Epigeic Collembola in winter wheat under organic, integrated and conventional farm management regimes. *Agriculture, Ecosystems & Environment*, **83**, 95-110.

Anderson, S., Bankier, A., Barrell, B., Debruijn, M., Coulson, A., Drouin, J., Eperon, I., Nierlich, D., Roe, B., Sanger, F., Schreier, P., Smith, A., Staden, R. & Young, I. (1981) Sequence and organization of the human mitochondrial genome. *Nature*, **290**, 457-465.

Arim, M. & Marquet, P. (2004) Intraguild predation: a widespread interaction related to species biology. *Ecology Letters*, **7**, 557-564.

Ayre, K. (2001) Effect of predator size and temperature on the predation of *Deroceras reticulatum* (Muller) (Mollusca) by carabid beetles. *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie*, **125**, 389-395.

Ayre, K. & Port, G.R. (1996) Carabid beetles recorded feeding on slugs in arable fields using ELISA. *Slug and Snail Pests in Agriculture.* British Crop Protection Council Symposium Proceedings. pp. 411-418.

Banerji, A. & Morin, P. (2009) Phenotypic plasticity, intraguild predation and anti-cannibal defences in an enigmatic polymorphic ciliate. *Functional Ecology*, **23**, 427-434.

Bascompte, J. (2009) Disentangling the Web of Life. *Science*, **325**, 416-419.

Bascompte, J. & Melian, C. (2005) Simple trophic modules for complex food webs. *Ecology*, **86**, 2868-2873.

Bascompte, J., Melian, C. & Sala, E. (2005) Interaction strength combinations and the overfishing of a marine food web. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 5443-5447.

Basky, Z. & Fónagy, A. (2003) Glutenin and gliadin contents of flour derived from wheat infested with different aphid species. *Pest Management Science*, **59**, 426-430.

Batista, R. & Oliveira, M.M. (2009) Facts and fiction of genetically engineered food. *Trends in Biotechnology*, **27**, 277-286.

Beckerman, A., Petchey, O. & Warren, P. (2006) Foraging biology predicts food web complexity. *Proc. Natl. Acad. Sci. U. S. A.*, **103**, 13745-13749.

Beer, E. & Heitefuss, R. (1981) Determination of economic thresholds and economic injury levels for monocotyl and dicotyl weeds in winter-wheat and winter-barley. 1. The methods of calculating threshold values in relation to economical and biological-techincal influences for the example winter-wheat. *Zeitschrift fur Pflanzenkranheiten und Pflanzenschutz - -Journal of Plant Diseases and Protection*, **88**, 65-85.

Bell, J., Bohan, D., Shaw, E. & Weyman, G. (2005) Ballooning dispersal using silk: world fauna, phylogenies, genetics and models. *Bulletin of Entomological Research*, **95**, 69-114.

Bell, J., Johnson, P., Hambler, C., Haughton, A., Smith, H., Feber, R., Tattersall, F., Hart, B., Manley, W. & Macdonald, D. (2002) Manipulating the abundance of *Lepthyphantes tenuis* (Araneae : Linyphiidae) by field margin management. *Agriculture Ecosystems & Environment*, **93**, 295-304.

Bell, J.R., King, R.A., Bohan, D.A. & Symondson, W.O.C. (2010) Spatial co-occurrence networks predict the feeding histories of polyphagous arthropod predators at field scales. *Ecography*, **33**, 64-72.

Bell, J., Traugott, M., Sunderland, K., Skirvin, D., Mead, A., Kravar-Garde, L., Reynolds, K., Fenlon, J. & Symondson, W. (2008) Beneficial links for the control of aphids: the effects of compost applications on predators and prey. *Journal of Applied Ecology*, **45**, 1266-1273.

Bengtsson, J., Ahnstrom, J. & Weibull, A. (2005) The effects of organic agriculture on biodiversity and abundance: a meta-analysis. *Journal of Applied Ecology*, **42**, 261-269.

Benton, T. (2007) Assessing Agriculture's Impact on Biodiversity. *Science*, **315**, 341-342.

Benton, T., Vickery, J. & Wilson, J. (2003) Farmland biodiversity: is habitat heterogeneity the key? *Trends in Ecology & Evolution*, **18**, 182-188.

Berlow, E., Neutel, A., Cohen, J., de Ruiter, P., Ebenman, B., Emmerson, M., Fox, J., Jansen, V., Jones, J., Kokkoris, G., Logofet, D., McKane, A., Montoya, J. & Petchey, O. (2004) Interaction strengths in food webs: issues and opportunities. *Journal of Animal Ecology*, **73**, 585-598.

Berson, D., Dunn, F. & Takao, M. (2002) Phototransduction by retinal ganglion cells that set the circadian clock. *Science*, **295**, 1070-1073.

Bianchi, F., Booij, C. & Tscharntke, T. (2006) Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proceedings of the Royal Society B-Biological Sciences*, **273**, 1715-1727.

Bilde, T. & Toft, S. (1997) Limited predation capacity by generalist arthropod predators on the cereal aphid, *Rhopalosiphum padi*. *Biological Agriculture & Horticulture*, **15**, 143-150.

Bilde, T. & Toft, S. (1998) Quantifying food limitation of arthropod predators in the field. *Oecologia*, **115**, 54-58.

Birkhofer, K., Wise, D. & Scheu, S. (2008) Subsidy from the detrital food web, but not microhabitat complexity, affects the role of generalist predators in an aboveground herbivore food web. *Oikos*, **117**, 494-500.

Bohan, D., Bohan, A., Glen, D., Symondson, W., Wiltshire, C. & Hughes, L. (2000) Spatial dynamics of predation by carabid beetles on slugs. *Journal of Animal Ecology*, **69**, 367-379.

Bommarco, R., Firle, S.O. & Ekbom, B. (2007) Outbreak suppression by predators depends on spatial distribution of prey. *Ecological Modelling*, **201**, 163-170.

Borer, E., Briggs, C., Murdoch, W. & Swarbrick, S. (2003) Testing intraguild predation theory in a field system: does numerical dominance shift along a gradient of productivity? *Ecology Letters*, **6**, 929-935.

Borer, E., Seabloom, E., Shurin, J., Anderson, K., Blanchette, C., Broitman, B., Cooper, S. & Halpern, B. (2005) What determines the strength of a trophic cascade? *Ecology*, **86**, 528-537.

Buddle, C. (2002) Interactions among young stages of the wolf spiders *Pardosa moesta* and *P. mackenziana* (Araneae : Lycosidae). *Oikos*, **96**, 130-136.

Burgio, G., Lanzoni, A., Accinelli, G. & Maini, S. (2008) Estimation of mortality by entomophages on exotic *Harmonia axyridis* versus native *Adalia bipunctata* in semi-field conditions in northern Italy. *Biocontrol*, **53**, 277-287.

Carapelli, A., Soto-Adames, F., Simon, C., Frati, F., Nardi, F. & Dallai, R. (2004) Secondary structure, high variability and conserved motifs for domain III of 12S rRNA in the Arthropleona (Hexapoda; Collembola). *Insect Molecular Biology*, **13**, 659-670.

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

Cardinale, B., Ives, A. & Inchausti, P. (2004) Effects of species diversity on the primary productivity of ecosystems: extending our spatial and temporal scales of inference. *Oikos*, **104**, 437-450.

Carpenter, S., Kitchell, J. & Hodgson, J. (1985) Cascading Trophic Interactions and Lake Productivity. *Bioscience*, **35**, 634-639.

Carson, R. (1962) Silent Spring. Houghton Mifflin, Boston, MA.

Chacon, J.M. & Heimpel, G.E. (2010) Density-dependent intraguild predation of an aphid parasitoid. *Oecologia*, **164**, 213-220.

Chambers, R. & Aikman, D. (1988) Quantifying the Effects of Predators on Aphid Populations. *Entomologia Experimentalis et Applicata*, **46**, 257-265.

Chang, G., Kareiva, P. (1999). The case for indigenous generalists in biological control. In *Theoretical Approaches to Biological Control* (Eds. Hawkin, B., & Cornell). Cambridge University Press, Cambridge.

Chapman, P. & Armstrong, G. (1997) Design and use of a time-sorting pitfall trap for predatory arthropods. *Agriculture Ecosystems & Environment*, **65**, 15-21.

Chapman, P., Armstrong, G. & McKinlay, R. (1999) Daily movements of *Pterostichus melanarius* between areas of contrasting vegetation density within crops. *Entomologia Experimentalis et Applicata*, **91**, 477-480.

Charnov, E. (1976) Optimal foraging, marginal value theorum. *Theoretical Population Biology*, **9**, 129-136.

Chen, Y., Giles, K., Payton, M. & Greenstone, M. (2000) Identifying key cereal aphid predators by molecular gut analysis. *Molecular Ecology*, **9**, 1887-1898.

Chiverton, P. (1986) Predator density manipulation and its effects on populations of *Rhopalosiphum padi* (Hom, Aphidae) in spring barley. *Annals of Applied Biology*, **109**, 49-60.

Chiverton, P. (1987) Effects of exclusion barriers and inclusion trenches on polyphagous and aphid specific predators in spring barley. *Journal of Applied Entomology-Zeitschrift fur Angewandte Entomologie*, **103**, 193-203.

Chiverton, P. & Sotherton, N. (1991) The effects on beneficial arthropods of the exclusion of herbicides from cereal crop edges. *Journal of Applied Ecology*, **28**, 1027-1039.

Cocquempot, C. & Chambon, J. (1990) Spider activity and its effect on the level of aphid populations in cereal biocenosis. *Revue d'Ecologie et de Biologie du Sol*, **27**, 205-209.

Colfer, R. & Rosenheim, J. (2001) Predation on immature parasitoids and its impact on aphid suppression. *Oecologia*, **126**, 292-304.

Costamagna, A., Landis, D. & Difonzo, C. (2007) Suppression of soybean aphid by generalist predators results in a trophic cascade in soybeans. *Ecological Applications*, **17**, 441-451.

Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., Oneill, R., Paruelo, J., Raskin, R., Sutton, P. & van den Belt, M. (1997) The value of the world's ecosystem services and natural capital. *Nature*, **387**, 253-260.

Cracraft, J. (1988) Deep-histoy biogeography - retrieving the historical pattern of evolving coastal biotas. *Sysytematic Zoology*, **37**, 221-236.

Crawley, M. J. (2007) The R Book. John Wiley & Sons Ltd, Chichester.

Cross, P. & Edwards-Jones, G. (2006) Variation in pesticide hazard from vegetable production in Great Britain from 1991 to 2003. *Pest Management Science*, **62**, 1058-1064.

Crowder, D.W. & Snyder, W.E. (2010) Eating their way to the top? Mechanisms underlying the success of invasive insect generalist predators. *Biological Invasions*, **12**, 2857-2876.

Daugherty, M., Harmon, J. & Briggs, C. (2007) Trophic supplements to intraguild predation. *Oikos*, **116**, 662-677.

Davey, J., Casey, C., Burgess, I. & Cable, J. (2007) DNA detection rates of host mtDNA in bloodmeals of human body lice (*Pediculus humanus* L., 1758). *Medical and Veterinary Entomology*, **21**, 293-296.

Davies, M. (1953) The contents of the crops of some British Carabid beetles. *Entomologists Monthly Magazine*, **89**, 18-23.

Dawson, N. (1965) A comparative study of the ecology of eight species of fenland Carabidae (Coleoptera). *The Journal of Animal Ecology*, **34**, 299-314.

de Keer, R. & Maelfait, J. (1988) Observations on the Life-Cycle of *Erigone atra* (Araneae, Erigoninae) in a Heavily Grazed Pasture. *Pedobiologia*, **32**, 201-212.

de los Monteros, A. (2003) Models of the primary and secondary structure for the 12S rRNA of birds: A guideline for sequence alignment. *DNA Sequence*, **14**, 241-256.

Dean, G. (1974) The overwintering and abundance of cereal aphids. *Annals of Applied Biology*, **76**, 1-7.

Dedryver, C., Le Ralec, A. & Fabre, F. (2010) The conflicting relationships between aphids and men: A review of aphid damage and control strategies. *Comptes Rendus Biologies*, **333**, 539-553.

Denno, R., Mitter, M., Langellotto, G., Gratton, C. & Finke, D. (2004) Interactions between a hunting spider and a web-builder: consequences of intraguild predation and cannibalism for prey suppression. *Ecological Entomology*, **29**, 566-577.

DEFRA (2010) Organic Statistics 2009 United Kingdom. Department for Environmental and Rural Affairs, London, UK.

DeRuiter, P., Moore, J., Zwart, K., Bouwman, L., Hassink, J., Bloem, J., Devos, J., Marinissen, J., Didden, W., Lebbink, G. & Brussaard, L. (1993) Simultion of nitrogen mineralization in the belowground food webs of 2 winter wheat fields. *Journal of Applied Ecology*, **30**, 95-106.

Diamond J (1998) Guns, Germs and Steel. Vintage, London. 480pp

Diehl, S. & Feissel, M. (2001) Intraguild prey suffer from enrichment of their resources: A microcosm experiment with ciliates. *Ecology*, **82**, 2977-2983.

Dinter, A. (1998) Intraguild predation between erigonid spiders, lacewing larvae and carabids. *Journal of Applied Entomology-Zeitschrift fur Angewandte Entomologie-Zeitschrift Fur Angewandte Entomologie*, **122**, 163-167.

Dixon, A. (1998) Aphid Ecology. Chapman & Hall, London, 157pp

Dixon, A. & Glen, D. (1971) Morph determination in Bird cherry oat aphid, *Rhopalosiphum padi* L. *Annals of Applied Biology*, **68**, 11-21

Dodd CS (2005). *Development and Optimisation of PCR-Based Techniques in Predator Gut Analysis*. PhD Thesis, Cardiff University, UK.

Donald, P. (1998) Changes in the abundance of invertebrates and plants on British Farmland. British Wildlife, **10**, 279-288.

Downie, I.S., Coulson, J.C. & Butterfield, J.E.L. (1996) Distribution and dynamics of surface-dwelling spiders across a pasture-plantation ecotone. *Ecography*, **19**, 29-40.

Duffield, S., Bryson, R., Young, J., Sylvester-Bradley, R. & Scott, R. (1997) The influence of nitrogen fertiliser on the population development of the cereal aphids *Sitobion avenae* (F.) and *Metopolophium dirhodum* (Wlk.) on field grown winter wheat. *Annals of Applied Biology*, **130**, 13-26.

Dunne, J., Williams, R. & Martinez, N. (2002) Food-web structure and network theory: The role of connectance and size. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 12917-12922.

Dutilleul, P. (1993) Modifying the t-test for assessing the correlation between two spatial processes. *Biometrics*, **49**, 305-314.

Easteal, S. (1981) The history of introductions of *Bufo marinus* (Amphibia; Anura) - a natural experiment in evolution. *Biological Journal of the Linnean Society*, **16**, 93-113.

Edwards, C., Sunderland, K. & George, K. (1979) Studies on Polyphagous Predators of Cereal Aphids. *Journal of Applied Ecology*, **16**, 811-823.

Eisenhauer, N., Hoersch, V., Moeser, J. & Scheu, S. (2010) Synergistic effects of microbial and animal decomposers on plant and herbivore performance. *Basic and Applied Ecology*, **11**, 23-34.

Emmerson, M. & Yearsley, J.M. (2004) Weak interactions, omnivory and emergent food-web properties. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **271**, 397-405.

Entwistle, J. & Dixon, A. (1987) Short-term forecasting of wheat yield loss caused by the grain aphid (*Sitobion avenae*) in summer. *Annals of Applied Biology*, **111**, 489-508.

Estes, J. & Palmisan, J. (1974) Sea Otters - Their Role in Structuring Nearshore Communities. *Science*, **185**, 1058-1060.

Elliott, N., Tao, F., Fuentes-Granados, R., Giles, K., Elliott, D., Greenstone, M., Shufran, K. & Royer, T. (2006) D-vac sampling for predatory arthropods in winter wheat. *Biological Control*, **38**, 325-330.

Fadl, A., Purvis, G. & Towey, K. (1996) The effect of time of soil cultivation on the incidence of *Pterostichus melanarius* (Illig) (Coleoptera: Carabidae) in arable land in Ireland. *Annales Zoologici Fennici*, **33**, 207-214.

FAO (2009) The state of Agricultural Commodity Markets. High Prices and the food crisis - experiences and lessons learned. United Nations Food and Agriculture Organization, Rome.

Feber, R., Bell, J., Johnson, P., Firbank, L. & Macdonald, D. (1998) The effects of organic farming on surface-active spider (Araneae) assemblages in wheat in southern England, UK. *Journal of Arachnology*, **26**, 190-202.

FiBL (2010) Organic agricultural land, share of total agricultural land, producers, Research Institute of Organic Agriculture FiBL, Frick, Switzerland

Fiebig, M., Poehling, H. & Borgemeister, C. (2004) Barley yellow dwarf virus, wheat, and *Sitobion avenae*: a case of trilateral interactions. *Entomologia Experimentata et Applicata.*, **110**, 11-21.

Fievet, V., Dedryver, C., Plantegenest, M., Simon, J. & Outreman, Y. (2007) Aphid colony turn-over influences the spatial distribution of the grain aphid *Sitobion avenae* over the wheat growing season. *Agricultural and Forest Entomology*, **9**, 125-134.

Finke, D. & Denno, R. (2004) Predator diversity dampens trophic cascades. *Nature*, **429**, 407-410.

Finke, D. & Denno, R. (2005) Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. *Ecology Letters*, **8**, 1299-1306.

Foelix, R. (1996). The Biology of Spiders. Oxford University Press, NY, 340pp

Folz, H., Wilder, S., Persons, M. & Rypstra, A. (2006) Effects of predation risk on vertical habitat use and foraging of *Pardosa milvina*. *Ethology*, **112**, 1152-1158.

Frampton, G. & Dorne, J. (2007) The effects on terrestrial invertebrates of reducing pesticide inputs in arable crop edges: a meta-analysis. *Journal of Applied Ecology*, **44**, 362-373.

Freier, S., Kierzek, R., Jaeger, J., Sugimoto, N., Caruthers, M., Neilson, T., Turner, D. (1986) Improved free-energy parameters for predictions of RNA duplex stability. *Proceedings of the National Academy Of Sciences of the United States of America*, **83**, 9373-9377.

Frihauf, J.C., Miller, S.D. & Alford, C.M. (2005) Imazamox rates, timings, and adjuvants affect imidazolinone-tolerant winter wheat cultivars. *Weed Technology*, **19**, 599-607.

Fuller, R.J., (1987) The changing extent and conservation interest of lowland grasslands in England and Wales - A review of grassland suveys 1930-84. *Biological Conservation*, **40**, 281-300.

Fuller, R.J., Gregory, R.D., Gibbons, D.W., Marchant, J.H., Wilson, J.D., Baillie, S.R. & Carter, N. (1995) Population declines and range contractions among lowland farmland birds in Britain. *Conservation Biology*, **9**, 1425-1441.

Gabriel, D., Carver, S.J., Durham, H., Kunin, W.E., Palmer, R.C., Sait, S.M., Stagl, S. & Benton, T.G. (2009) The spatial aggregation of organic farming in England and its underlying environmental correlates. *Journal of Applied Ecology*, **46**, 323-333.

Gariepy, T., Kuhlmann, U., Gillott, C. & Erlandson, M. (2007) Parasitoids, predators and PCR: the use of diagnostic molecular markers in biological control of Arthropods. *Journal of Applied Entomology-Zeitschrift fur Angewandte Entomologie*, **131**, 225-240.

Gaskell, G., Bauer, M.W., Durant, J. & Allum, N.C. (1999) Worlds apart? The reception of genetically modified foods in Europe and the US. *Science*, **285**, 384-387.

Gause, I. (1934) The Struggle for Existence. Williams & Wilkins, Baltimore, MD.

Gavish-Regev, E., Lubin, Y. & Coll, M. (2008) Linking keystone species and functional groups: a new operational definition of the keystone species concept. *Ecological Entomology*, **33**, 202-212.

Giannopoulos, G., Pulleman, M.M. & van Groenigen, J.W. (2010) Interactions between residue placement and earthworm ecological strategy affect aggregate turnover and N_2O dynamics in agricultural soil. *Soil Biology & Biochemistry*, **42**, 618-625.

Glover, J., Culman, S., DuPont, S., Broussard, W., Young, L., Mangan, M., Mai, J., Crews, T., DeHaan, L., Buckley, D., Ferris, H., Turner, R., Reynolds, H. & Wyse, D. (2010) Harvested perennial grasslands provide ecological benchmarks for agricultural sustainability. *Agriculture, Ecosystems and Environment*, **137**, 3-12.

Godfray, H.C.J., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Nisbett, N., Pretty, J., Robinson, S., Toulmin, C. & Whiteley, R. (2010) The future of the global food system. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365**, 2769 -2777.

Goggin, F. (2007) Plant-aphid interactions: molecular and ecological perspectives. *Current Opinion in Plant Biology.*, **10**, 399-408.

Goodwin, B.J., Jones, C.G., Schauber, E.M. & Ostfeld, R.S. (2005) Limited dispersal and heterogeneous predation risk synergistically enhance persistence of rare prey. *Ecology*, **86**, 3139-3148.

Gotelli, N.J. and G.L. Entsminger. 2004. EcoSim: Null models software for ecology. Version 7. Acquired Intelligence Inc. & Kesey-Bear. Jericho, VT 05465. http://garyentsminger.com/ecosim/index.htm.

Grafen, A. & Hails, R. (2002) Modern Statistics for the Life Sciences. Oxford University Press, Oxford.

Gravesen, E. & Toft, S. (1987) Grass Fields as Reservoirs for Polyphagous Predators (Arthropoda) of Aphids (Homopt, Aphididae). *Journal of Applied Entomology-Zeitschrift fur Angewandte Entomologie*, **104**, 461-473.

Greenslade, P. (1963) Daily rhythms of locomotor activity in some Carabidae. *Entomologia Experimentalis et Applicata.*, **6**, 171-180.

Greenstone, M., Rowley, D., Weber, D., Payton, M. & Hawthorne, D. (2007) Feeding mode and prey detectability half-lives in molecular gut-content analysis: an example with two predators of the Colorado potato beetle. *Bulletin of Entomological Research*, **97**, 201-209.

Greenstone, M.H., Szendrei, Z., Payton, M.E., Rowley, D.L., Coudron, T.C. & Weber, D.C. (2010) Choosing natural enemies for conservation biological control: use of the prey detectability half-life to rank key predators of Colorado potato beetle. *Entomologia Experimentalis et Applicata*, **136,** 97-107.

Grez, A., Rivera, P. & Zaviez, T. (2007) Foliar and ground-foraging predators of aphids associated with alfalfa crops in Chile: Are they good or bad partners? *Biocontrol Science and Technology*, **17**, 1071-1077.

Griffiths, G., Winder, L., Holland, J., Thomas, C. & Williams, E. (2007) The representation and functional composition of carabid and staphylinid beetles in different field boundary types at a farm-scale. *Biological Conservation*, **135**, 145-152.

Griffiths, G., Holland, J., Bailey, A. & Thomas, M. (2008) Efficacy and economics of shelter habitats for conservation biological control. *Biological Control*, **45**, 200-209.

Gross, T. (2009) Generalized models reveal stabilizing factors in food webs. *Science*, **325**, 1341.

Gross, T., Edwards, A.M. & Feudel, U. (2009) The invisible niche: Weakly density-dependent mortality and the coexistence of species. *Journal of Theoretical Biology*, **258**, 148-155.

Guy, A., Bohan, D., Powers, S. & Reynolds, A. (2008) Avoidance of conspecific odour by carabid beetles: a mechanism for the emergence of scale-free searching patterns. *Animal Behaviour*, **76**, 585-591.

Hairston, N.G., Smith, F.E., Slobodkin, L.B. (1960) Community structure, population control and competition. *American Naturalist*, **94**, 421-425

Halaj, J. & Wise, D. (2001) Terrestrial trophic cascades: How much do they trickle? *American Naturalist*, **157**, 262-281.

Halaj, J. & Wise, D. (2002) Impact of a detrital subsidy on trophic cascades in a terrestrial grazing food web. *Ecology*, **83**, 3141-3151.

Harper, G., King, R., Dodd, C., Harwood, J., Glen, D., Bruford, M. & Symondson, W. (2005) Rapid screening of invertebrate predators for multiple prey DNA targets. *Molecular Ecology*, **14**, 819-827.

Harper, G., Sheppard, S., Harwood, J., Read, D., Glen, D., Bruford, M. & Symondson, W. (2006) Evaluation of temperature gradient gel electrophoresis for the analysis of prey DNA within the guts of invertebrate predators. *Bulletin of Entomological Research*, **96**, 295-304.

Harwood, J. (2008) Are sweep net sampling and pitfall trapping compatible with molecular analysis of predation? *Environmental Entomology*, **37**, 990-995.

Harwood, J. & Obrycki, J. (2005) Web-construction behavior of linyphiid spiders (Araneae, Linyphiidae): Competition and co-existence within a generalist predator guild. *Journal of Insect Behavior*, **18**, 593-607.

Harwood, J. & Obrycki, J. (2007) Web-site selection strategies of linyphiid spiders in alfalfa: implications for biological control. *Biocontrol*, **52**, 451-467.

Harwood, J., Sunderland, K. & Symondson, W. (2003) Web-location by linyphiid spiders: prey-specific aggregation and foraging strategies. *Journal of Animal Ecology*, **72**, 745-756.

Harwood, J., Sunderland, K. & Symondson, W. (2004) Prey selection by linyphiid spiders: molecular tracking of the effects of alternative prey on rates of aphid consumption in the field. *Molecular Ecology*, **13**, 3549-3560.

Harwood, J., Sunderland, K. & Symondson, W. (2001) Living where the food is: web location by linyphiid spiders in relation to prey availability in winter wheat. *Journal of Applied Ecology*, **38**, 88-99.

Harwood, J., Sunderland, K. & Symondson, W. (2005) Monoclonal antibodies reveal the potential of the tetragnathid spider *Pachygnatha degeeri* (Araneae : Tetragnathidae) as an aphid predator. *Bulletin of Entomological Research*, **95**, 161-167.

Harwood, J., Yoo, H., Greenstone, M., Rowley, D. & O'Neil, R. (2009) Differential impact of adults and nymphs of a generalist predator on an exotic invasive pest demonstrated by molecular gut-content analysis. *Biological Invasions*, **11**, 895-903.

Hassell, M.P., Comins, H.N. & May, R.M. (1991) Spatial structure and chaos in insect population dynamics. *Nature*, **353**, 255-258.

Hastings, A. (2001) Transient dynamics and persistence of ecological systems. *Ecology Letters*, **4**, 215-220.

Hatteland, B., Haukeland, S., Roth, S., Brurberg, M. & Symondson, W. (in prep.) Spatiotemporal dynamics of predation by carabid beetles (Carabidae) on nematode infected and uninfected slugs in the field.

Hatten, T., Bosque-Perez, N. & Johnson-Maynard, J. (2007) Tillage differentially affects the capture rate of pitfall traps for three species of carabid beetles. *Entomologia Experimentalis et Applicata*, **124**, 177-187.

Haughton, A., Bell, J., Boatman, N. & Wilcox, A. (1999) The effects of different rates of the herbicide glyphosate on spiders in arable field margins. *Journal of Arachnology*, **27**, 249-254.

Hengeveld, R. (1980) Polyphagy, Oligophagy and Food Specialization in Ground Beetles (Coleoptera, Carabidae). *Netherlands Journal of Zoology*, **30**, 564-584.

Herberstein, M. (1998) Web placement in sympatric linyphiid spiders (Arachnida, Araneae): individual foraging decisions reveal inter-specific competition. *Acta Oecologica-International Journal of Ecology*, **19**, 67-71.

Hickson, R., Simon, C., Copper, A., Spicer, G., Sullivan, J. & Penny, D. (1996) Conserved sequence motifs, alignment, and secondary structure for the third domain of animal 12S rRNA. *Molecular Biology and Evolution*, **13**, 150-169.

Hirsch, R.L. (2008) Mitigation of maximum world oil production: Shortage scenarios. *Energy Policy*, **36**, 881-889.

Hoffmann, A., Reynolds, K., Nash, M. & Weeks, A. (2008) A high incidence of parthenogenesis in agricultural pests. *Proceedings of the Royal Society B-Biological Sciences*, **275**, 2473-2481.

Hole, D., Perkins, A., Wilson, J., Alexander, I., Grice, P. & Evans, A. (2005) Does organic farming benefit biodiversity? *Biological Conservation*, **122**, 113-130.

Holland, J.M., Begbie, M., Birkett, T., Southway, S., Thomas, S.R., Alexander, C.J. & Thomas, C.F.G. (2004) The spatial dynamics and movement of *Pterostichus melanarius* and *P madidus* (Carabidae) between and within arable fields inthe UK. *International Journal of Ecology and Environmental Sciences*, **30**, 35–53.

Holland, J., Oaten, H., Southway, S. & Moreby, S. (2008) The effectiveness of field margin enhancement for cereal aphid control by different natural enemy guilds. *Biological Control*, **47**, 71-76.

Holland, J., Birkett, T. & Southway, S. (2009) Contrasting the farm-scale spatio-temporal dynamics of boundary and field overwintering predatory beetles in arable crops. *Biocontrol*, **54**, 19-33.

Holland, J. & Reynolds, C. (2003) The impact of soil cultivation on arthropod (Coleoptera and Araneae) emergence on arable land. *Pedobiologia*, **47**, 181-191.

Holling, C.S. (1959a) The components of predation as revealed by a study of small-mammal predation of the European pine sawfly. *Canadian Entomologist*, **91**, 293–320.

Holling, C.S. (1959b) Some characteristics of simple types of predation and parasitism. *Canadian Entomologist*, **91**, 385–398.

Holt, R. & Huxel, G. (2007) Alternative prey and the dynamics of intraguild predation: Theoretical perspectives. *Ecology*, **88**, 2706-2712.

Holt, R. & Lawton, J. (1994) The Ecological Consequences of Shared Natural Enemies. *Annual Review of Ecology and Systematics*, **25**, 495-520.

Holt, R. & Polis, G. (1997) A theoretical framework for intraguild predation. *American Naturalist*, **149**, 745-764.

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

Hortal, J., Roura-Pascual, N., Sanders, N. & Rahbek, C. (2010) Understanding (insect) species distributions across spatial scales. *Ecography*, **33**, 51-53.

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

Hothorn, T., F., B. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50,** 346-363.

Hubbell, S. (2005) Neutral theory in community ecology and the hypothesis of functional equivalence. *Functional Ecology*, **19**, 166-172.

Hutchinson, G. (1957) Population studies - animal ecology and demography - concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, **22**, 415-427.

Ilbery, B. & May, D. (2010) Clustering and the spatial distribution of organic farming in England and Wales. Area, doi: 10.1111/j.1475-4762.2010.00953.x

Imhasly, P. & Nentwig, W. (1995) Habitat management in winter-wheat and evaluation of subsequent spider predation on insect pests. *Acta Oecologica-International Journal of Ecology*, **16**, 389-403.

Ings, T.C., Montoya, J.M., Bascompte, J., Bluthgen, N., Brown, L., Dormann, C.F., Edwards, F., Figueroa, D., Jacob, U., Jones, J.I., Lauridsen, R.B., Ledger, M.E., Lewis, H.M., Olesen, J.M., Veen, F.F.V., Warren, P.H. & Woodward, G. (2009) Review: Ecological networks - beyond food webs. *Journal of Animal Ecology*, **78**, 253-269.

IPCC, 2007: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds. Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor & Miller H.L.), Cambridge University Press, Cambridge.

Janssen, A., Montserrat, M., HilleRisLambers, R., De Roos, A., Pallini, A. & Sabelis, M. (2006) Intraguild predation usually does not disrupt biological control. *Trophic and Guild Interactions in Biological Control.* Progress in Biological Control. (eds. Brodeur J. & Boivin G.), pp. 21-44. Springer, Dordrecht.

Janssen, A., Sabelis, M., Magalhaes, S., Montserrat, M. & Van der Hammen, T. (2007) Habitat structure affects intraguild predation. *Ecology*, **88**, 2713-2719.

Janssens, J. & DeClercq, R. (1990) Observations on Carabidae, Staphylinidae and Araneae as predators of cereal aphids in winter wheat. *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent*, **55**, 471-475.

Johnston, R. & Bishop, G. (1987) Economic injury levels and economicthresholds for cereal aphids (Homoptera, Aphididae) on Spring-planted wheat. *Journal of Economic Entomology*, **80**, 478-482.

Joly, D.O. & Patterson, B.R. (2003) Use of selection indices to model the functional response of predators. *Ecology*, **84**, 1635-1639.

Ke, X. & Scheu, S. (2008) Earthworms, Collembola and residue management change wheat (*Triticum aestivum*) and herbivore pest performance (Aphidina : *Rhophalosiphum padi*). *Oecologia*, **157**, 603-617.

Keeling, M.J., Wilson, H.B. & Pacala, S.W. (2000) Reinterpreting Space, Time Lags, and Functional Responses in Ecological Models. *Science*, **290**, 1758-1761.

Keeling, M.J., Woolhouse, M.E.J., Shaw, D.J., Matthews, L., Chase-Topping, M., Haydon, D.T., Cornell, S.J., Kappey, J., Wilesmith, J. & Grenfell, B.T. (2001) Dynamics of the 2001 UK foot and mouth epidemic: Stochastic dispersal in a heterogeneous landscape. *Science*, **294**, 813-817.

Kendal, W.S. (2004) Taylor's ecological power law as a consequence of scale invariant exponential dispersion models. *Ecological Complexity*, **1**, 193-209.

Kielty, J., Allen-Williams, L. & Underwood, N. (1999) Prey preferences of six species of Carabidae (Coleoptera) and one Lycosidae (Araneae) commonly found in UK arable crop fields. *Journal of Applied Entomology-Zeitschrift fur Angewandte Entomologie*, **123**, 193-200.

King, R., Moreno-Ripoll, R., Agustí, N., Shayler, S., Bell, J., Bohan, D. & Symondson, W. (2010a) Multiplex reactions for the molecular detection of predation on pest and nonpest invertebrates in agroecosystems. *Molecular Ecology Resources*,. doi: 10.1111/j.1755-0998.2010.02913.x

King, R., Read, D., Traugott, M. & Symondson, W. (2008) Molecular analysis of predation: a review of best practice for DNA-based approaches. *Molecular Ecology*, **17**, 947-963.

King, R., Vaughan, I., Bell, J., Bohan, D. & Symondson, W. (2010b) Prey choice by carabid beetles feeding on an earthworm community analysed using species- and lineage-specific PCR primers. *Molecular Ecology*, **19**, 1721-1732.

Koch, R.L. (2003) The multicolored Asian lady beetle, *Harmonia axyridis*: A review of its biology, uses in biological control, and non-target impacts. *Journal of Insect Science*, **3.32**, 1-16

Koch, R.L. & Galvan, T.L. (2008) Bad side of a good beetle: the North American experience with *Harmonia axyridis*. *Biocontrol*, **53**, 23-35.

Kogan, M. (1998) Integrated pest management: Historical perspectives and contemporary developments. *Annual Review of Entomology*, **43**, 243-270.

Kromp, B. (1999) Carabid beetles in sustainable agriculture: a review on pest control efficacy, cultivation impacts and enhancement. *Agriculture Ecosystems & Environment*, **74**, 187-228.

Kruse, P., Toft, S. & Sunderland, K. (2008) Temperature and prey capture: opposite relationships in two predator taxa. *Ecological Entomology*, **33**, 305-312.

Kuusk, A. & Agustí, N. (2007) Group-specific primers for DNA-based detection of springtails (Hexapoda : Collembola) within predator gut contents. *Molecular Ecology Resources*, **8**, 678-681.

Kuusk, A. & Ekbom, B. (2010) Lycosid spiders and alternative food: Feeding behavior and implications for biological control. *Biological Control*, **55**, 20-26.

Lang, A. (2000) The pitfalls of pitfalls: a comparison of pitfall trap catches and absolute density estimates of epigeal invertebrate predators in arable land. *Anzeiger fur Schadlingskunde (Journal of Pest Science)*, **73**, 99-106.

Lang, A. (2003) Intraguild interference and biocontrol effects of generalist predators in a winter wheat field. *Oecologia*, **134**, 144-153.

Larsson, H. (2005) A crop loss model and economic thresholds for the grain aphid, *Sitobion avenae* (F.), in winter wheat in southern Sweden. *Crop Protection*, **24**, 397-405.

Lawton, J. (1999) Are there general laws in ecology? *Oikos*, **84**, 177-192.

Lefcort, H., Ben-Ami, F. & Heller, J. (2006) Terrestrial snails use predator-diet to assess danger. *Journal of Ethology*, **24**, 97-102.

Legendre, P. & Legendre, L. (1998) Numerical Ecology (Second Edition). Elsevier, New York, NY, 853pp.

Lemke, A. & Poehling, H. (2002) Sown weed strips in cereal fields: overwintering site and "source" habitat for *Oedothorax apicatus* (Blackwall) and *Erigone atra* (Blackwall) (Araneae : Erigonidae). *Agriculture Ecosystems & Environment*, **90**, 67-80.

Leslie, T., Werf, W., Bianchi, F. & Honěk, A. (2009) Population dynamics of cereal aphids: influence of a shared predator and weather. *Agricultural and Forest Entomology*, **11**, 73-82.

Levin, S. (1992) The Problem of Pattern and Scale in Ecology. *Ecology*, **73**, 1943-1967.

Lindeman, R.L. (1942) The trophic-dynamic aspect of ecology. *Ecology*, **23**, 399-417.

Llewellyn, K.S., Loxdale, H.D., Harrington, R., Brookes, C.P., Clark, S.J. & Sunnucks, P. (2003) Migration and genetic structure of the grain aphid (*Sitobion avenae*) in Britain related to climate and clonal fluctuation as revealed using microsatellites. *Molecular Ecology*, **12**, 21-34.

Losey, J. & Denno, R. (1998) Positive predator-predator interactions: Enhanced predation rates and synergistic suppression of aphid populations. *Ecology*, **79**, 2143-2152.

Losey, J. & Denno, R. (1999) Factors facilitating synergistic predation: The central role of synchrony. *Ecological Applications*, **9**, 378-386.

Losey, J. & Vaughan, M. (2006) The economic value of ecological services provided by insects. *Bioscience*, **56**, 311-323.

Lotka, A. (1920) Undamped oscillations derived from the law of mass action. *Journal of the American Chemical Society*, **42** (8), 1595-1599.

Lövei, G. & Sunderland, K. (1996) Ecology and behavior of ground beetles (Coleoptera: Carabidae). *Annual Review of Entomology*, **41**, 231-256.

Loveland, P. J., Chambers, B. & Black, H. (2000). The Impact of Changing Farming Practices on Sustainablity of Soil in England and Wales. MAFF, London.

Lu, Y., Wu, K., Jiang, Y., Xia, B., Li, P., Feng, H., Wyckhuys, K.A.G. & Guo, Y. (2010) Mirid Bug Outbreaks in Multiple Crops Correlated with Wide-Scale Adoption of *Bt* Cotton in China. *Science*, **328**, 1151-1154.

Lucas, E. (2005) Intraguild predation among aphidophagous predators. *European Journal of Entomology*, **102**, 351-363.

Luff, M. (1974) Adult and Larval Feeding Habits of *Pterostichus madidus* (F) (Coleoptera-Carabidae). *Journal of Natural History*, **8**, 403-409.

Luff, M. (1978) Diel activity patterns of some field Carabidae. *Ecological Entomology*, **3**, 53-62.

Lundgren, J.G. & Weber, D.C. (2010) Changes in digestive rate of a predatory beetle over its larval stage: Implications for dietary breadth. *Journal of Insect Physiology*, **56**, 431-437.

Lupwayi, N., Clayton, G., Hanson, K., Rice, W. & Biederbeck, V.(2004) Populations and functional diversity of bacteria associated with barley, wheat and canola roots. *Canadian Journal of Soil Science*, **84**, 245-254.

Lushai, G., Sherratt, T.N., David, O., De Barro, P.J. & Maclean, N. (1997) Host selection by winged summer females of the aphid Sitobion avenae. *Entomologia Experimentalis et Applicata*, **85**, 199-209.

MacArthur, R. & Pianka, E. (1966) On optimal use of a patchy environment. *American Naturalist*, **100**, 603-609.

Macfadyen, S., Gibson, R., Polaszek, A., Morris, R.J., Craze, P.G., Planqué, R., Symondson, W.O. & Memmott, J. (2009) Do differences in food web structure between organic and conventional farms affect the ecosystem service of pest control? *Ecology Letters*, **12**, 229-238.

MacMillan, K., Blok, V., Young, I., Crawford, J. & Wilson, M. (2006) Quantification of the slug parasitic nematode *Phasmarhabditis hermaphrodita* from soil samples using real time qPCR. *International Journal for Parasitology*, **36**, 1453-1461.

Madsen, M., Terkildsen, S. & Toft, S. (2004) Microcosm studies on control of aphids by generalist arthropod predators: Effects of alternative prey. *Biocontrol*, **49**, 483-504.

Magalhães, S., Tudorache, C., Montserrat, M., van Maanen, R., Sabelis, M. & Janssen, A. (2005) Diet of intraguild predators affects antipredator behavior in intraguild prey. *Behavioral Ecology*, **16**, 364-370.

Mair, J. & Port, G. (2002) The influence of mucus production by the slug, *Deroceras reticulatum*, on predation by *Pterostichus madidus* and *Nebria brevicollis* (Coleoptera : Carabidae). *Biocontrol Science and Technology*, **12**, 325-335.

Malthus, T.R. (1798) An Essay on the Principle of Population. J. Johnson, London.

Manly, B.F.J. (1997a) A method for the estimation of parameters for natural stage-structured populations. *Researches on Population Ecology*, **39**, 101-111.

Manly, B.F.J. (1997b) Randomization, Bootstrap and Monte Carlo Methods in Biology. Hartnolls Ltd., Bodmin, 399pp.

Manly, B., Miller, P. & Cook, L. (1972) Analysis of a selective predation experiment. *American Naturalist*, **106**, 719-736.

Mann, J., Tatchell, G., Dupuch, M., Harrington, R., Clark, S. & McCartney, H. (1995) Movement of apterous *Sitobion avenae* (Homoptera, Aphididae) in response to leaf disturbances caused by wind and rain. *Annals of Applied Biology*, **126**, 417-427.

Mansour, F. & Heimbach, U. (1993) Evaluation of lycosid, micryphantid and linyphiid spiders as predators of *Rhopalosiphum padi* (Hom.; Aphididae) and their functional response to prey density - laboratory experiments. *Entomophaga*, **38**, 79-87.

Marcussen, B., Axelsen, J. & Toft, S. (1999) The value of two Collembola species as food for a linyphiid spider. *Entomologia Experimentalis et Applicata*, **92**, 29-36.

McCann, K., Hastings, A. & Huxel, G. (1998) Weak trophic interactions and the balance of nature. *Nature*, **395**, 794-798.

McKemey, A., Symondson, W. & Glen, D. (2003) Predation and prey size choice by the carabid beetle *Pterostichus melanarius* (Coleoptera: Carabidae): the dangers of extrapolating from laboratory to field. *Bulletin of Entomological Research*, **93**, 227-234.

McLaughlin, A. & Mineau, P. (1995) The impact of agricultural practices on biodiversity. *Agriculture, Ecosystems & Environment*, **55**, 201-212.

McNabb, D., Halaj, J. & Wise, D. (2001) Inferring trophic positions of generalist predators and their linkage to the detrital food web in agroecosystems: a stable isotope analysis. *Pedobiologia*, **45**, 289-297.

Meadows, D.H., Meadows, D.L. & Randers, J. (1972) The Limits to Growth. Universe Books, New York, NY, 208pp

Meadows, D.H., Meadows, D.L. & Randers, J. (2004) The Limits to Growth: The thirty-year update. Earthscan Ltd., London, 362pp

Moore, J.W. (2010) The End of the Road? Agricultural Revolutions in the Capitalist World-Ecology, 1450–2010. *Journal of Agrarian Change*, **10**, 389-413.

Mikkelsen, R.L. & Bruulsema, T.W. (2005) Fertilizer use for horticultural crops in the US during the 20th century. *Horttechnology*, **15**, 24-30.

Miñarro, M., Espadaler, X., Melero, V.X. & Suárez- Álvarez, V. (2009) Organic versus conventional management in an apple orchard: effects of fertilization and tree-row management on ground-dwelling predaceous arthropods. *Agricultural and Forest Entomology*, **11**, 133-142.

Miyashita, T., Takada, M. & Shimazaki, A. (2003) Experimental evidence that aboveground predators are sustained by underground detritivores. *Oikos*, **103**, 31-36.

Morin, P. (1999) Productivity, intraguild predation, and population dynamics in experimental food webs. *Ecology*, **80**, 752-760.

Muller, C. & Brodeur, J. (2002) Intraguild predation in biological control and conservation biology. *Biological Control*, **25**, 216-223.

Muller, C., Fellowes, M. & Godfray, H. (2005) Relative importance of fertiliser addition to plants and exclusion of predators for aphid growth in the field. *Oecologia*, **143**, 419-427.

Muller, C. & Godfray, H. (1999) Indirect interactions in aphid-parasitoid communities. *Researches on Population Ecology*, **41**, 93-106.

Naranjo, S. & Hagler, J. (2001) Toward the quantification of predation with predator gut immunoassays: A new approach integrating functional response behavior. *Biological Control*, **20**, 175-189.

Newton, C. & Dixon, A. (1990) Pattern of growth in weight of alate and apterous nymphs of the English grain aphid, *Sitobion avenae*. *Entomologia Experimentalis et Applicata*, **55**, 231-238.

Nicolas, H. (2004) Using remote sensing to determine of the date of a fungicide application on winter wheat. *Crop Protection*, **23**, 853-863.

Nyffeler, M. & Benz, G. (1988) Prey and predatory importance of micryphantid spiders in winter wheat fields and hay meadows. *Journal of Applied Entomology*, **105**, 190-197.

Nyffeler, M. & Sunderland, K. (2003) Composition, abundance and pest control potential of spider communities in agroecosystems: a comparison of European and US studies. *Agriculture Ecosystems & Environment*, **95**, 579-612.

Oksanen, L., Fretwell, S., Arruda, J. & Niemela, P. (1981) Exploitation Ecosystems in Gradients of Primary Productivity. *American Naturalist*, **118**, 240-261.

Östman, O., Ekbom, B. & Bengtsson, J. (2001a) Landscape heterogeneity and farming practice influence biological control. *Basic and Applied Ecology*, **2**, 365-371.

Östman, O., Ekbom, B., Bengtsson, J. & Weibull, A.C. (2001b) Landscape complexity and farming practice influence the condition of polyphagous carabid beetles. *Ecological Applications*, **11**, 480-488.

Östman, O., Ekbom, B. & Bengtsson, J. (2003) Yield increase attributable to aphid predation by ground-living polyphagous natural enemies in spring barley in Sweden. *Ecological Economics*, **45**, 149-158.

Overton, M. (1996) Agricultural Revolution in England: The Transformation of the Agrarian Economy 1500-1850, Cambridge University Press, Cambridge, UK, 274pp

Page, R.D.M., Cruickshank, R. & Johnson, K.P. (2002) Louse (Insecta: Phthiraptera) mitochondrial 12S rRNA secondary structure is highly variable. *Insect Molecular Biology*, **11**, 361-369.

Passmore, A., Jarman, S., Swadling, K., Kawaguchi, S., McMinn, A. & Nicol, S. (2006) DNA as a Dietary Biomarker in Antarctic Krill, *Euphausia superba*. *Marine Biotechnology*, **8**, 686-696.

Pearce, S. & Zalucki, M. (2006) Do predators aggregate in response to pest density in agroecosystems? Assessing within-field spatial patterns. *Journal of Applied Ecology*, **43**, 128-140.

Penney, M. (1966) Studies on certain aspects of the ecology of *Nebria brevicollis* (F.) (Coleoptera : Carabidae). *Journal of Animal Ecology.*, **35**, 505-512.

Perry, J.N. (1995) Spatial Analysis by Distance Indices. *Journal of Animal Ecology*, **64**, 303-314.

Perry, J. (1998) Measures of spatial pattern for counts. *Ecology*, **79**, 1008-1017.

Perry, J., Liebhold, A., Rosenberg, M., Dungan, J., Miriti, M., Jakomulska, A. & Citron-Pousty, S. (2002) Illustrations and guidelines for selecting statistical methods for quantifying spatial pattern in ecological data. *Ecography*, **25**, 578-600.

Perry, J., Winder, L., Holland, J. & Alston, R. (1999) Red-blue plots for detecting clusters in count data. *Ecology Letters*, **2**, 106-113.

Petchey, O.L., Beckerman, A.P., Riede, J.O. & Warren, P.H. (2008) Size, foraging, and food web structure. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 4191-4196.

Phipps, R.H. & Park, J.R. (2002) Environmental benefits of genetically modified crops: Global and European perspectives on their ability to reduce pesticide use. *Journal of Animal and Feed Sciences*, **11**, 1-18.

Pianka, E.R. (1973) The Structure of Lizard Communities. *Annual Review of Ecology and Systematics*, **4**, 53-74.

Pimentel, D. (2009) Energy Inputs in Food Crop Production in Developing and Developed Nations. *Energies*, **2**, 1-24.

Pimm, S. & Lawton, J. (1978) Feeding on More Than One Trophic Level. *Nature*, **275**, 542-544.

Pinheiro, J. C. & Bates, D. M. (2000) Mixed-Effects Models in S and S-PLUS. Springer, New York, 528pp

Pinheiro, J. C., Bates, D. M., DebRoy, S., Sarkar D., & The R Core Development Team (2009) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-96.

Polis, G. & Holt, R. (1992) Intraguild Predation - the Dynamics of Complex Trophic Interactions. *Trends in Ecology & Evolution*, **7**, 151-154.

Polis, G., Myers, C. & Holt, R. (1989) The Ecology and Evolution of Intraguild Predation - Potential Competitors That Eat Each Other. *Annual Review of Ecology and Systematics*, **20**, 297-330.

Polis, G. & Strong, D. (1996) Food web complexity and community dynamics. *American Naturalist*, **147**, 813-846.

Prasad, R. & Snyder, W. (2004) Predator interference limits fly egg biological control by a guild of ground-active beetles. *Biological Control*, **31**, 428-437.

Prasad, R. & Snyder, W. (2006) Polyphagy complicates conservation biological control that targets generalist predators. *Journal of Applied Ecology*, **43**, 343-352.

Purvis, G. & Fadl, A. (1996) Emergence of Carabidae (Coleoptera) from pupation: A technique for studying the 'productivity' of carabid habitats. *Annales Zoologici Fennici*, **33**, 215-223.

R Core Development Team (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing,Vienna. 409pp

Raun, W.R. & Johnson, G.V. (1999) Improving nitrogen use efficiency for cereal production. *Agronomy Journal*, **91**, 357-363.

Read, D., Sheppard, S., Bruford, M., Glen, D. & Symondson, W. (2006) Molecular detection of predation by soil micro-arthropods on nematodes. *Molecular Ecology*, **15**, 1963-1972.

Remén, C., Krueger, M. & Cassel-Lundhagen, A. (2010) Successful analysis of gut contents in fungal-feeding oribatid mites by combining body-surface washing and PCR. *Soil Biology and Biochemistry*, **42**, 1952-1957.

Rieger, S., Richner, W., Streit, B., Frossard, E. & Liedgens, M. (2008) Growth, yield, and yield components of winter wheat and the effects of tillage intensity, preceding crops, and N fertilisation. *European Journal of Agrnomy*, **28**, 405-411.

Ritchie, E. & Johnson, C. (2009) Predator interactions, mesopredator release and biodiversity conservation. *Ecology Letters*, **12**, 982-998.

Risch, S., Andow, D. & Altieri, M. (1983) Agroecosystem diversity and pest-control - data, tentative conclusions, and new research directions. *Environmental Entomology*, **12**, 625-629.

Roberts, M. (1996) *Spiders of Britain and Northern Europe* (3rd Edition). Harper Collins, London, UK, 456pp.

Robinson, R. & Sutherland, W. (2002) Post-war changes in arable farming and biodiversity in Great Britain. *Journal of Applied Ecology*, **39**, 157-176.

Root, R. (1973) Organization of a Plant-Arthropod Association in Simple and Diverse Habitats - Fauna of Collards (Brassica-Oleracea). *Ecological Monographs*, **43**, 95-120.

Rosenheim, J. & Corbett, A. (2003) Omnivory and the indeterminacy of predator function: Can a knowledge of foraging behavior help? *Ecology*, **84**, 2538-2548.

Rosenheim, J., Kaya, H., Ehler, L., Marois, J. & Jaffee, B. (1995) Intraguild Predation among Biological-Control Agents - Theory and Evidence. *Biological Control*, **5**, 303-335.

Rosenheim, J., Wilhoit, L. & Armer, C. (1993) Influence of Intraguild Predation among Generalist Insect Predators on the Suppression of an Herbivore Population. *Oecologia*, **96**, 439-449.

Roy, H., Baverstock, J., Ware, R., Clark, S., Majerus, M., Baverstock, K. & Pell, J. (2008) Intraguild predation of the aphid pathogenic fungus Pandora neoaphidis by the invasive coccinellid Harmonia axyridis. *Ecological Entomology*, **33**, 175-182.

Rudolf, V. (2007) The interaction of cannibalism and omnivory: Consequences for community dynamics. *Ecology*, **88**, 2697-2705.

Samu, F., Sunderland, K., Topping, C. & Fenlon, J. (1996b) A spider population in flux: Selection and abandonment of artificial web-sites and the importance of intraspecific interactions in *Lepthyphantes tenuis* (Araneae: Linyphiidae) in wheat. *Oecologia*, **106**, 228-239.

Samu, F., Voros, G. & Botos, E. (1996a) Diversity and community structure of spiders of alfalfa fields and grassy field margins in south Hungary. *Acta Phytopathologica et Entomologica Hungarica*, **31**(3-4), 253-266.

Salmon, S. & Ponge, J. (2001) Earthworm excreta attract soil springtails: laboratory experiments on *Heteromurus Nitidus* (Collembola: Entomobryidae). *Soil Biology and Biochemistry*, **33**, 1959-1969.

Samu, F. & Szinetár, C. (2002) On the nature of agrobiont spinders. *Journal of Arachnology*, **30**, 389-402.

Schernthaner, J., Milne, R. & Kaplan, H. (2002) Characterization of a novel insect digestive DNase with a highly alkaline pH optimum. *Insect Biochemistry and Molecular Biology*, **32**, 255-263.

Scheu, S. (2001) Plants and generalist predators as links between the below-ground and above-ground system. *Basic and Applied Ecology*, **2**, 3-13.

Schmidt, M., Lauer, A., Purtauf, T., Thies, C., Schaefer, M. & Tscharntke, T. (2003) Relative importance of predators and parasitoids for cereal aphid control. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270**, 1905-1909.

Schmidt, M., Thies, C., Nentwig, W. & Tscharntke, T. (2008) Contrasting responses of arable spiders to the landscape matrix at different spatial scales. *Journal of Biogeography*, **35**, 157-166.

Schmidt, M. & Tscharntke, T. (2005) The role of perennial habitats for Central European farmland spiders. *Agriculture, Ecosystems & Environment*, **105**, 235-242.

Schmitz, O. (2007) Predator diversity and trophic interactions. *Ecology*, **88**, 2415-2426

Schmitz, O., Hamback, P. & Beckerman, A. (2000) Trophic cascades in terrestrial systems: A review of the effects of carnivore removals on plants. *American Naturalist*, **155**, 141-153.

Schoenly, K., Cohen, J., Heong, K., Litsinger, J., Barrion, A. & Arida, G.(2010) Fallowing did not disrupt invertebrate fauna in Philippine low-pesticide irrigated rice fields. *Journal of Applied Ecology*, **47**, 593-602.

Settle, W.H., Ariawan, H., Astuti, E.T., Cahyana, W., Hakim, A.L., Hindayana, D., Lestari, A.S., Pajarningsih & Sartanto. (1996) Managing tropical rice pests through conservation of generalist natural enemies and alternative prey. *Ecology*, **77**, 1975-1988.

Shakya, S., Weintraub, P. & Coll, M. (2009) Effect of pollen supplement on intraguild predatory interactions between two omnivores: The importance of spatial dynamics. *Biological Control*, **50**, 281-287.

Sheppard, S. & Harwood, J. (2005) Advances in molecular ecology: tracking trophic links through predator-prey food-webs. *Functional Ecology*, **19**, 751-762.

Sheppard, S., Bell, J., Sunderland, K., Fenlon, J., Skervin, D. & Symondson, W. (2005) Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. *Molecular Ecology*, **14**, 4461-4468.

Shurin, J., Borer, E., Seabloom, E., Anderson, K., Blanchette, C., Broitman, B., Cooper, S. & Halpern, B. (2002) A cross-ecosystem comparison of the strength of trophic cascades. *Ecology Letters*, **5**, 785-791.

Sih, A. & Christensen, B. (2001) Optimal diet theory: when does it work, and when and why does it fail? *Animal Behaviour*, **61**, 379-390.

Sih, A., Englund, G. & Wooster, D. (1998) Emergent impacts of multiple predators on prey. *Trends in Ecology & Evolution*, **13**, 350-355.

Simberloff, D. & Stiling, P. (1996) How risky is biological control? *Ecology*, **77**, 1965-1974.

Simon, J., Blackman, R. & LeGallic, J. (1991) Local variability in the life cycle of the Bird cherry oat aphid, *Rhapalosiphum padi* (Homopera, Aphididae) in Western France. *Bulletin of Entomological Research*, **81**, 315-322.

Simon, J., Stoeckel, S. & Tagu, D. (2010) Evolutionary and functional insights into reproductive strategies of aphids. *Comptes Rendus Biologies*, **333**, 488-496.

Sigsgaard, L. (2007) Early season natural control of the brown planthopper, *Nilaparvata lugens*: the contribution and interaction of two spider species and a predatory bug. *Bulletin of Entomological Research*, **97**, 533-544.

Slobodkin LB, Smith F, Hairston Sr. NG (1967) Regulation of terrestrial ecosystems and the implied balance of nature. *American Naturalist*, **101**, 109-124.

Smil, V. (2002) Nitrogen and food production: Proteins for human diets. *Ambio*, **31**, 126-131.

Snyder, W. & Ives, A. (2001) Generalist predators disrupt biological control by a specialist parasitoid. *Ecology*, **82**, 705-716.

Snyder, W. & Ives, A. (2003) Interactions between specialist and generalist natural enemies: Parasitoids, predators, and pea aphid biocontrol. *Ecology*, **84**, 91-107.

Snyder, W., Snyder, G., Finke, D. & Straub, C. (2006) Predator biodiversity strengthens herbivore suppression. *Ecology Letters*, **9**, 789-796.

Snyder, W. & Wise, D. (2001) Contrasting trophic cascades generated by a community of generalist predators. *Ecology*, **82**, 1571-1583.

Soberon, J. & Nakamura, M. (2009) Niches and distributional areas: Concepts, methods, and assumptions. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 19644-19650.

Sopp, P. & Wratten, S. (1986) Rates of consumption of cereal aphids by some polyphagous predatos in the laboratory. *Entomologia Experimentalis et Applicata*, **41**, 69-73.

Stoate, C., Boatman, N., Borralho, R., Carvalho, C., de Snoo, G. & Eden, P. (2001) Ecological impacts of arable intensification in Europe. *Journal of Environmental Management*, **63**, 337-365.

Straub, C. & Snyder, W. (2006) Species identity dominates the relationship between predator biodiversity and herbivore suppression. *Ecology*, **87**, 277-282.

Straub, C., Finke, D. & Snyder, W. (2008) Are the conservation of natural enemy biodiversity and biological control compatible goals? *Biological Control*, **45**, 225-237.

Sunderland, K. (1975) Diet of Some Predatory Arthropods in Cereal Crops. *Journal of Applied Ecology*, **12**, 507-515.

Sunderland, K. (1999) Mechanisms underlying the effects of spiders on pest populations. *Journal of Arachnology*, **27**, 308-316.

Sunderland, K., Fraser, A. & Dixon, A. (1986) Distribution of linyphiid spiders in relation to capture of prey in cereal fields. *Pedobiologia*, **29**, 367-375.

Sunderland, K., Crook, N., Stacey, D. & Fuller, B. (1987) A Study of Feeding by Polyphagous Predators on Cereal Aphids Using ELISA and Gut Dissection. *Journal of Applied Ecology*, **24**, 907-933.

Sunderland, K. & Samu, F. (2000) Effects of agricultural diversification on the abundance, distribution, and pest control potential of spiders: a review. *Entomologia Experimentalis et Applicata*, **95**, 1-13.

Sunderland, K. & Topping, C. (1993) The spatial dynamics of linyphiid spiders in winter wheat. *Memoirs of the Queensland Museum*, **33**, 639-644.

Sunderland, K. & Vickerman, G. (1980) Aphid feeding by some polyphagous predators in relation to aphid density in cereal fields. *Journal of Applied Ecology*, **17**, 389-396.

Symondson, W. (2002) Molecular identification of prey in predator diets. *Molecular Ecology*, **11**, 627-641.

Symondson, W., Glen, D., Erickson, M., Liddell, J. & Langdon, C. (2000) Do earthworms help to sustain the slug predator *Pterostichus melanarius* (Coleoptera : Carabidae) within crops? Investigations using monoclonal antibodies. *Molecular Ecology*, **9**, 1279-1292.

Symondson, W., Glen, D., Wiltshire, C., Langdon, C. & Liddell, J. (1996) Effects of cultivation techniques and methods of straw disposal on predation by Pterostichus melanarius (Coleoptera: Carabidae) upon slugs (Gastropoda: Pulmonata) in an arable field. *Journal of Applied Ecology*, **33**, 741-753.

Symondson, W., Glen, D., Ives, A., Langdon, C. & Wiltshire, C. (2002b) Dynamics of the relationship between a generalist predator and slugs over five years. *Ecology*, **83**, 137-147.

Symondson, W. & Liddell, J. (1993) A Monoclonal-Antibody for the Detection of Arionid Slug Remains in Carabid Predators. *Biological Control*, **3**, 207-214.

Symondson, W., Sunderland, K. & Greenstone, M. (2002a) Can generalist predators be effective biocontrol agents? *Annual Review of Entomology*, **47**, 561-594.

Taylor, L. (1984) Assessing and interpreting the spatial distributions of insect populations. *Annual Review Of Entomology*, **29**, 321-357.

Thomas, C.F., Brain, P. & Jepson, P. (2003) Aerial activity of linyphiid spiders: modelling dispersal distances from meteorology and behaviour. *Journal of Applied Ecology*, **40**, 912-927.

Thomas, C.F., Parkinson, L., Griffiths, G., Garcia, A. & Marshall, E. (2001) Aggregation and temporal stability of carabid beetle distributions in field and hedgerow habitats. *Journal of Applied Ecology*, **38**, 100-116.

Thomas, C.F, Parkinson, L. & Marshall, E. (1998) Isolating the components of activity-density for the carabid beetle *Pterostichus melanarius* in farmland. *Oecologia*, **116**, 103-112.

Thomas, R., Glen, D. & Symondson, W. (2008) Prey detection through olfaction by the soil-dwelling larvae of the carabid predator *Pterostichus melanarius*. *Soil Biology & Biochemistry*, **40**, 207-216.

Thomas, R., Harwood, J., Glen, D. & Symondson, W. (2009) Tracking predator density dependence and subterranean predation by carabid larvae on slugs using monoclonal antibodies. *Ecological Entomology*, **34**, 569-579.

Thompson, J., Higgins, D. & Gibosn, T. (1994) ClustalW - Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673-4680.

Thorbek, P. & Bilde, T. (2004) Reduced numbers of generalist arthropod predators after crop management. *Journal of Applied Ecology*, **41**, 526-538.

Thorbek, P., Sunderland, K. & Topping, C. (2003) Eggsac development rates and phenology of agrobiont linyphiid spiders in relation to temperature. *Entomologia Experimentalis et Applicata*, **109**, 89-100.

Tilman, D. (2004) Niche tradeoffs, neutrality, and community structure: A stochastic theory of resource competition, invasion, and community assembly. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 10854 -10861.

Tobin, P.C. & Bjørnstad, O.N. (2003) Spatial dynamics and cross-correlation in a transient predator–prey system. *Journal of Animal Ecology*, **72**, 460-467.

Topping, C. & Sunderland, K. (1998) Population dynamics and dispersal of *Lepthyphantes tenuis* in an ephemeral habitat. *Entomologia Experimentalis et Applicata*, **87**, 29-41.

Traugott, M., Bell, J., Broad, G., Powell, W., Veen, F., Vollhardt, I. & Symondson, W. (2008) Endoparasitism in cereal aphids: molecular analysis of a whole parasitoid community. *Molecular Ecology*, **17**, 3928-3938.

Traugott, M. & Symondson, W. (2008) Molecular analysis of predation on parasitized hosts. *Bulletin of Entomological Research*, **98**, 223-231.

Troedsson, C., Simonelli, P., Nagele, V., Nejstgaard, J.C. & Frischer, M.E. (2009) Quantification of copepod gut content by differential length amplification quantitative PCR (dla-qPCR). *Marine Biology*, **156**, 253-259.

Tscharntke, T., Bommarco, R., Clough, Y., Crist, T., Kleijn, D., Rand, T., Tylianakis, J., van Nouhuys, S. & Vidal, S. (2008) Conservation biological control and enemy diversity on a landscape scale (Reprinted from Biol. Control, vol 43, pg 294-309, 2007). *Biological Control*, **45**, 238-253.

Valentini, A., Miquel, C., Nawaz, M.A., Bellemain, E., Coissac, E., Pompanon, F., Gielly, L., Cruaud, C., Nascetti, G., Wincker, P., Swenson, J.E. & Taberlet, P. (2009) New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the trnL approach. *Molecular Ecology Resources*, **9**, 51-60.

van der Heijden, M., Klironomos, J., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A. & Sanders, I. (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, **396**, 69-72.

Vandermeer, J.(1972) Niche Theory. *Annual Review of Ecology and Systematics*, **3**, 107-132.

Venables, W. & Ripley, B. (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York. 512pp

Verch, G., Kaechele, H., Hoeltl, K., Richter, C. & Fuchs, C. (2009) Comparing the profitability of tillage methods in Northeast Germany-A field trial from 2002 to 2005. *Soil & Tillage Research*, **104**, 16-21.

Vickerman, G. & Wratten, S. (1979) The biology and pest status of cereal aphids (Hemiptera: Aphididae) in Europe: a review. *Bulletin of Entomological Research*, **69**, 1-32.

Volterra, V. (1926) Variazioni e fluttuazioni del numero d'individui in specie animali conviventi. *Memorie della Reale Accademia Nazionale dei Lincei*, **2**, 31-11.

von Berg, K., Traugott, M., Symondson, W.O.C. & Scheu, S. (2008) Impact of abiotic factors on predator-prey interactions: DNA-based gut content analysis in a microcosm experiment. *Bulletin of Entomological Research*, **98**, 257-261.

von Berg, K., Thies, C., Tscharntke, T. & Scheu, S. (2010) Changes in herbivore control in arable fields by detrital subsidies depend on predator species and vary in space. *Oecologia*, **163**, 1033-1042.

Wallin, H. & Ekbom, B. (1994) Influence of hunger level and prey densities on movement patterns in three species of *Pterostichus* beetles (Coleoptera, Carabidae). *Environmental Entomology*, **23**, 1171-1181.

Watt, A. & Dixon, A.(1981) The role of cereal growth stages and crowding in the induction of alatae in *Sitobion avenae* and its consequences for population growth. *Ecological Entomology*, **6**, 441-447.

Webb, D.M. & Moore, W.S. (2005) A phylogenetic analysis of woodpeckers and their allies using 12S, Cyt b, and COI nucleotide sequences (class Aves; order Piciformes). *Molecular Phylogenetics and Evolution*, **36**, 233-248.

Weber, D., Ferro, D., Buonaccorsi, J. & Hazzard, R. (1994) Disrupting spring colonization of Colorado potato beetle to nonrotated potato fields. *Entomologia Experimentalis et Applicata*, **73**, 39-50.

Weber, D. & Lundgren, J. (2009) Detection of Predation Using qPCR: Effect of Prey Quantity, Elapsed Time, Chaser Diet, and Sample Preservation on Detectable Quantity of Prey DNA. *Journal of Insect Science*, **9**, 1-12.

Webster, R. & Oliver, M. (1989) Optimal interpolation and isarithmic mapping of soil properties VI. Disjunctive kriging and mapping the conditional-probability. *Journal of Soil Science*, **40**, 497-512.

Wiens, J., Crist, T., With, K. & Milne, B. (1995) Fractal Patterns of Insect Movement in Microlandscape Mosaics. *Ecology*, **76**, 663-666.

Wilby, A., Villareal, S., Lan, L., Heong, K. & Thomas, M. (2005) Functional benefits of predator species diversity depend on prey identity. *Ecological Entomology*, **30**, 497-501.

Williams, R.J. & Martinez, N.D. (2000) Simple rules yield complex food webs. *Nature*, **404**, 180-183.

Wilson, P.J. (1999) The effect of nitrogen on populations of rare arable plants in Britain. *Aspects of Applied Biology*, **55**, 93-100.

Winder, L., Alexander, C., Holland, J., Symondson, W., Perry, J. & Woolley, C. (2005) Predatory activity and spatial pattern: the response of generalist carabids to their aphid prey. *Journal of Animal Ecology*, **74**, 443-454.

Winder, L., Alexander, C., Holland, J., Woolley, C. & Perry, J. (2001) Modelling the dynamic spatio-temporal response of predators to transient prey patches in the field. *Ecology Letters*, **4**, 568-576.

Winder, L., Hirst, D., Carter, N., Wratten, S. & Sopp, P. (1994) Estimating predation of the grain aphid *Sitobion avenae* by polyphagous predators. *Journal of Applied Ecology*, **31**, 1-12.

Winder, L., Perry, J. & Holland, J. (1999) The spatial and temporal distribution of the grain aphid *Sitobion avenae* in winter wheat. *Entomologia Experimentalis et Applicata*, **93**, 277-290.

Wise, D. (2006) Cannibalism,food limitation, intraspecific competition and the regulation of spider populations. *Annual Review of Entomology*, **51**, 441-465.

Wise, D., Moldenhauer, D. & Halaj, J. (2006) Using stable isotopes to reveal shifts in prey consumption by generalist predators. *Ecological Applications*, **16**, 865-876.

Wissinger, S.A. (1997) Cyclic Colonization in Predictably Ephemeral Habitats: A Template for Biological Control in Annual Crop Systems. *Biological Control*, **10**, 4-15.

Wootton, J.T. & Emmerson, M. (2005) Measurement of interaction strength in nature. *Annual Review of Ecology Evolution and Systematics*, **36**, 419-444.

Wratten, S. (1975) Nature of effects of aphids *Sitobion* avenae and *Metoplophium dirhodum* on growth of wheat. *Annals of Applied Bology*, **79**, 27-34.

Wratten, S., Watt, A., Carter, N. & Entwistle, J. (1990) Economic consequences of pesticide use for grain aphid control on Winter-Wheat in 1984 in England. *Crop Protection*, **9**, 73-78.

Woodward, G. & Hildrew, A. (2002) Body-size determinants of niche overlap and intraguild predation within a complex food web. *Journal of Animal Ecology*, **71**, 1063-1074.

Wu, K., Lu, Y., Feng, H., Jiang, Y. & Zhao, J. (2008) Suppression of Cotton Bollworm in Multiple Crops in China in Areas with *Bt* Toxin-Containing Cotton. *Science*, **321**, 1676-1678.

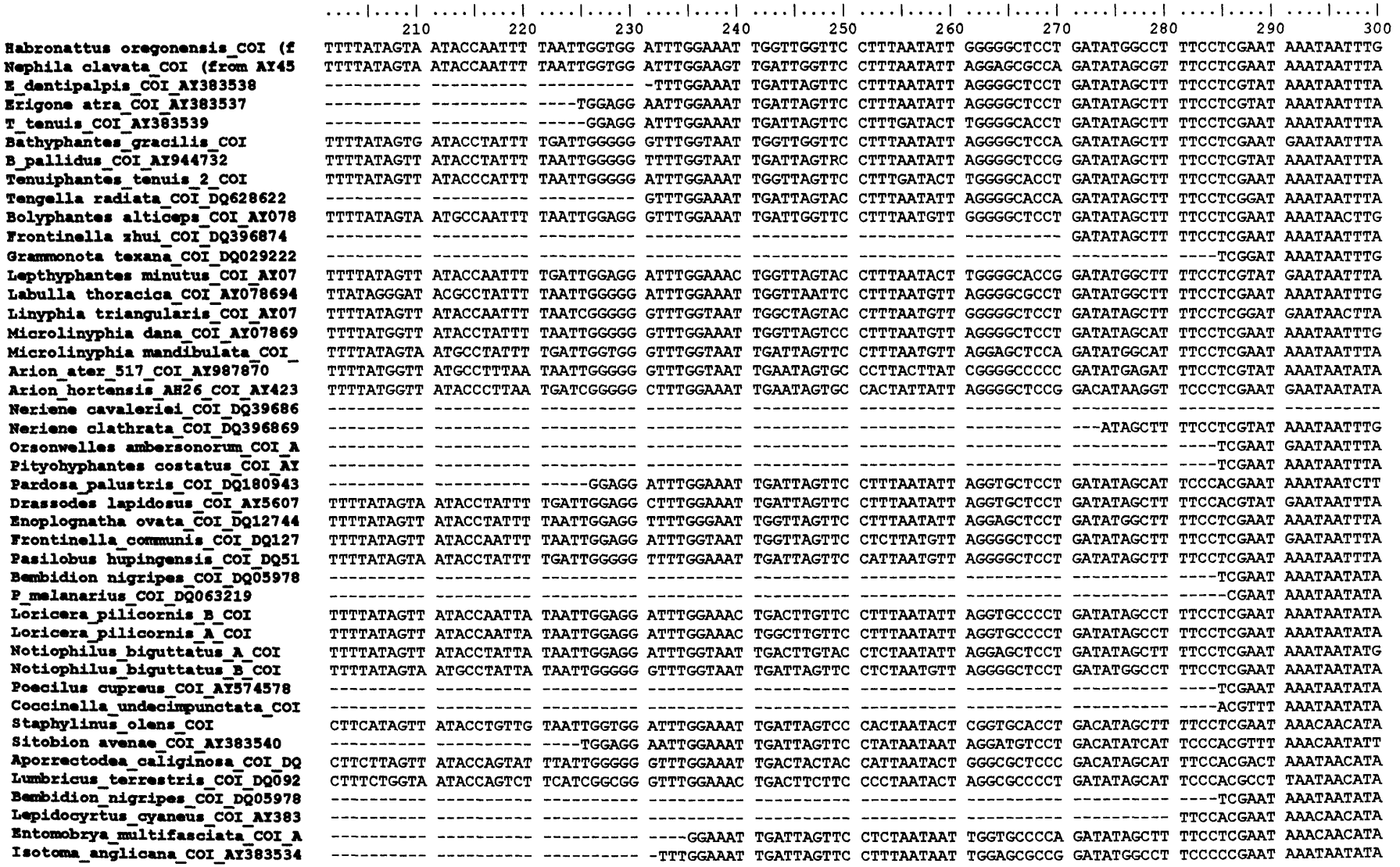
Zaidi, R., Jaal, Z., Hawkes, N., Hemingway, J. & Symondson, W. (1999) Can multiple-copy sequences of prey DNA be detected amongst the gut contents of invertebrate predators? *Molecular Ecology*, **8**, 2081-2087.

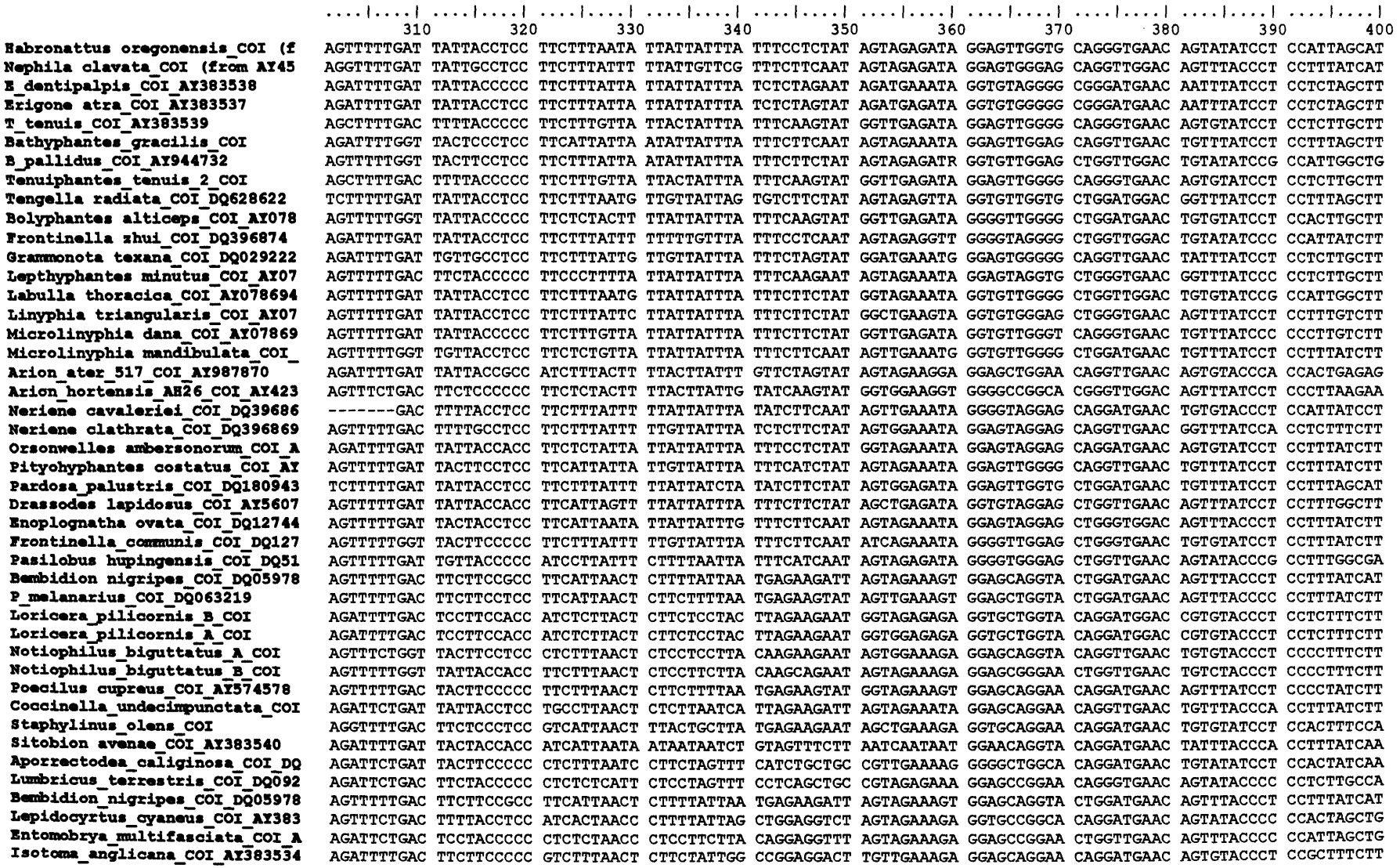
Zohary, D. & Hopf, M. (1993) Domestication of Plants in the Old World: The Origin and Spread of Cultivated Plants in West Asia, Europe, and the Nile Valley (Second Edition). Oxford University Press, Oxford, 328pp.

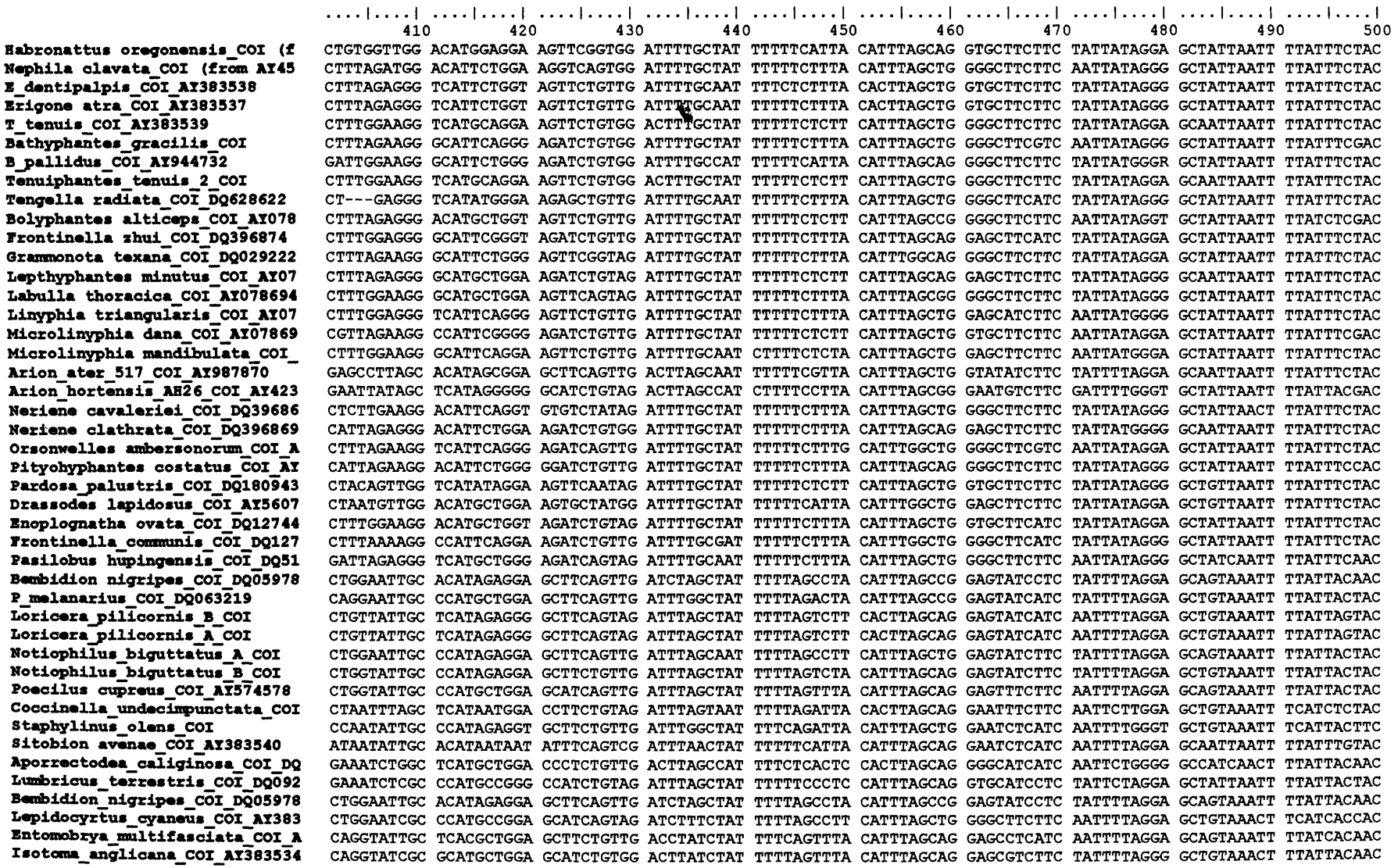
Alignment: D:\Sequences\Alignments\COI_alignments\Wheat_aln.fas

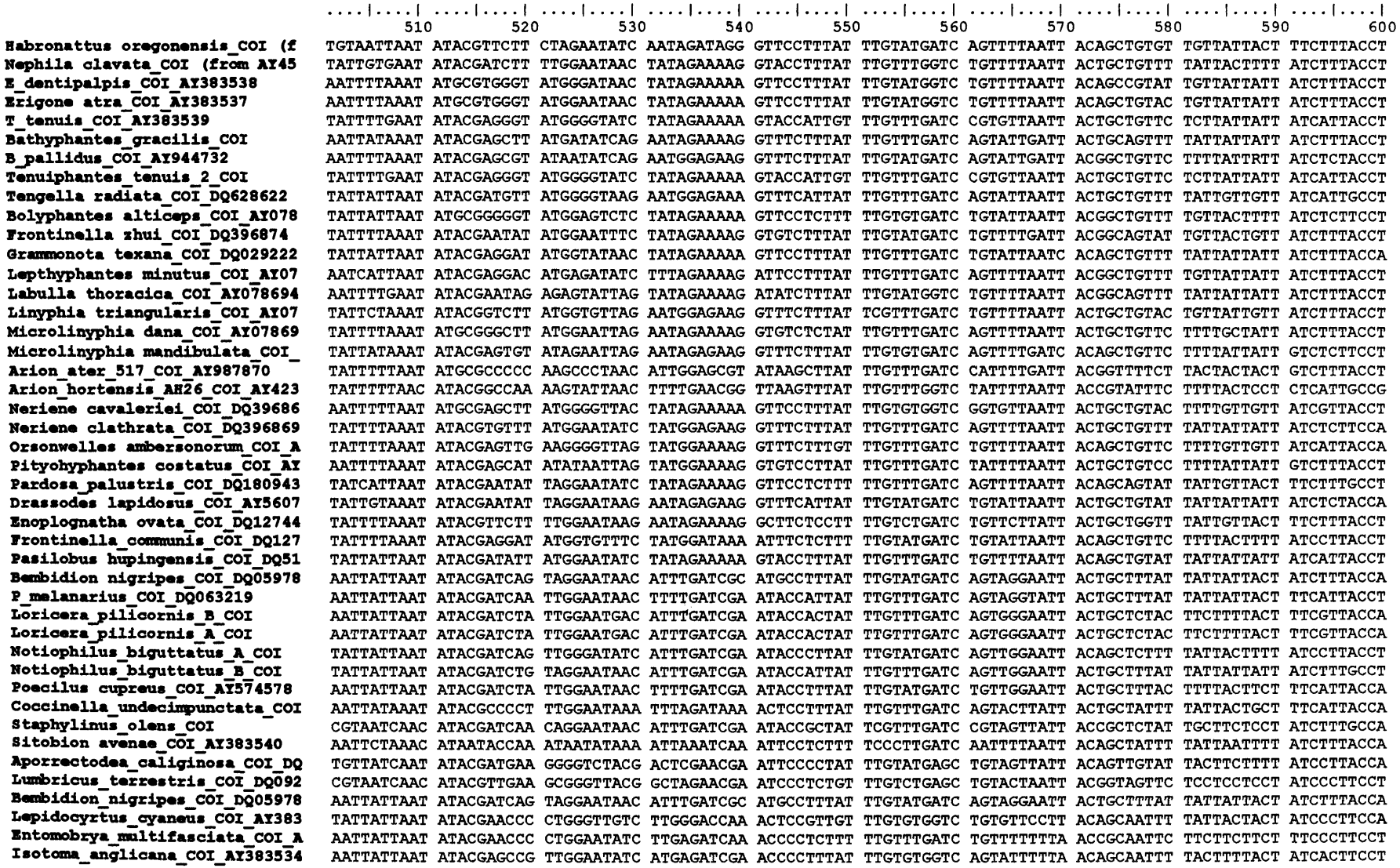
```
                                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                         10         20         30         40         50         60         70         80         90        100
Habronattus oregonensis_COI (f   ATTGAGTTAC TGCGATGATT ATATTCTACT AATCATAAGG ATATTGGAAC TTTGTATTTA ATTTTTGGTG CTTGAGCTGC TATAGTGGGT ACTGCTATAA
Nephila clavata_COI (from AY45   ------TTAC TGCGATGATT ATATTCAACA AATCATAAAG ATATTGGAAC ATTATATTTA GTTTTTGGGG CTTGAGCAGC TATAGTTGGT ACTGCAATAA
E_dentipalpis_COI_AY383538       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Erigone atra_COI_AY383537        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
T_tenuis_COI_AY383539            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Bathyphantes_gracilis_COI        ---------- ---------- ---------- ---------- ---------- ---------- -----TGGTG CTTGAGCAGC TATAGTTGGG ACTGCTATAA
B_pallidus_COI_AY944732          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------ATAA
Tenuiphantes_tenuis_2_COI        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -CTGCTATAA
Tengella radiata_COI_DQ628622    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Bolyphantes alticeps_COI_AY078   ---------- ---------- ---------- ---------- -------TTC TTTATATTTT ATTTTTGGGG CGTGAGCTGC TATGGTGGGG ACTGCTATAA
Frontinella zhui_COI_DQ396874    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Grammonota texana_COI_DQ029222   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Lepthyphantes minutus_COI_AY07   ---------- ---------- ---------- ---------- -------AAC TTTATATTTT ATTTTTGGGG CTTGGTCTGC TATAGTAGGG ACTGCTATAA
Labulla thoracica_COI_AY078694   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Linyphia triangularis_COI_AY07   ---------- ---------- ---------- ---------- --------AC TTTGTATTTC ATTTTTGGAG CTTGAGCTGC CATGGTTGGT ACAGCTATAA
Microlinyphia dana_COI_AY07869   ---------- ---------- ---------- ---------- -------AAC TTTGTATTTT ATTTTTGGGG CTTGAGCTGC CATAGTAGGA ACTGCTATAA
Microlinyphia mandibulata_COI_   ---------- ---------- ---------- AATCATAAAG ATATTGGAAC TTTATATTTT ATTTTTGGTG CTTGAGCTGC TATGGTAGGT ACCGCTATAA
Arion_ater_517_COI_AY987870      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------T
Arion_hortensis_AH26_COI_AY423   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ACCGGCTTGT
Neriene cavaleriei_COI_DQ39686   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Neriene clathrata_COI_DQ396869   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Orsonwelles ambersonorum_COI_A   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Pityohyphantes costatus_COI_AY   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Pardosa palustris_COI_DQ180943   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Drassodes lapidosus_COI_AY5607   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---GATCTTC -ATAGTAGGA ACAGCTATAA
Enoplognatha ovata_COI_DQ12744   ---------- ---------- ---------- ---------- ---------- ---------- ---------- -------TGC TATAGTAGGT ACAGCTATAA
Frontinella_communis_COI_DQ127   ---------- ---------- ---------- ---------- ---------- ---------- ATTTTTGGGG CATGATCTGC TATAGTGGGT ACGGCCATAA
Pasilobus hupingensis_COI_DQ51   ---------- ---------- ---------- ---------- ---------- ---------- ---------- -TTGATCTGC TATAGTAGGG ACAGCTATAA
Bembidion nigripes_COI_DQ05978   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P_melanarius_COI_DQ063219        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Loricera_pilicornis_B_COI        ---------- ---------- ---------- ---------- ---------- ---------- -------GAG CTTGATCAGG AATAGTGGGA ACCTCCCTAA
Loricera_pilicornis_A_COI        ---------- ---------- ---------- ---------- ---------- ---------- -------GAG CTTGATCAGG AATAGTGGGA ACCTCCCTAA
Notiophilus_biguttatus_A_COI     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --TAGTTGGA ACTTCTTTAA
Notiophilus_biguttatus_B_COI     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -ATAGTGGGA ACTTCTTTAA
Poecilus cupreus_COI_AY574578    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Coccinella_undecimpunctata_COI   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Staphylinus_olens_COI            ---------- ---------- ---------- --TCATAAAG ATATTGGAAC ACTATACTTC ATTTTCGGAG TATGGTCAGG AATAGTAGGA ACTTCCCTCA
Sitobion avenae_COI_AY383540     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Aporrectodea_caliginosa_COI_DQ   ---------- ---------- ---------- ---------- -------AAC TTTATATTTT ATCCTCGGCG TCTGAGCCGG TATAGTCGGG GCCGGAATAA
Lumbricus_terrestris_COI_DQ092   ---------- ---------- ---------- ---------- -------AAC TCTATACTTC ATTCTTGGGG TGTGAGCTGG CATGGTGGGG GCCGGGATAA
Bembidion_nigripes_COI_DQ05978   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Lepidocyrtus_cyaneus_COI_AY383   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Entomobrya_multifasciata_COI_A   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Isotoma_anglicana_COI_AY383534   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
```

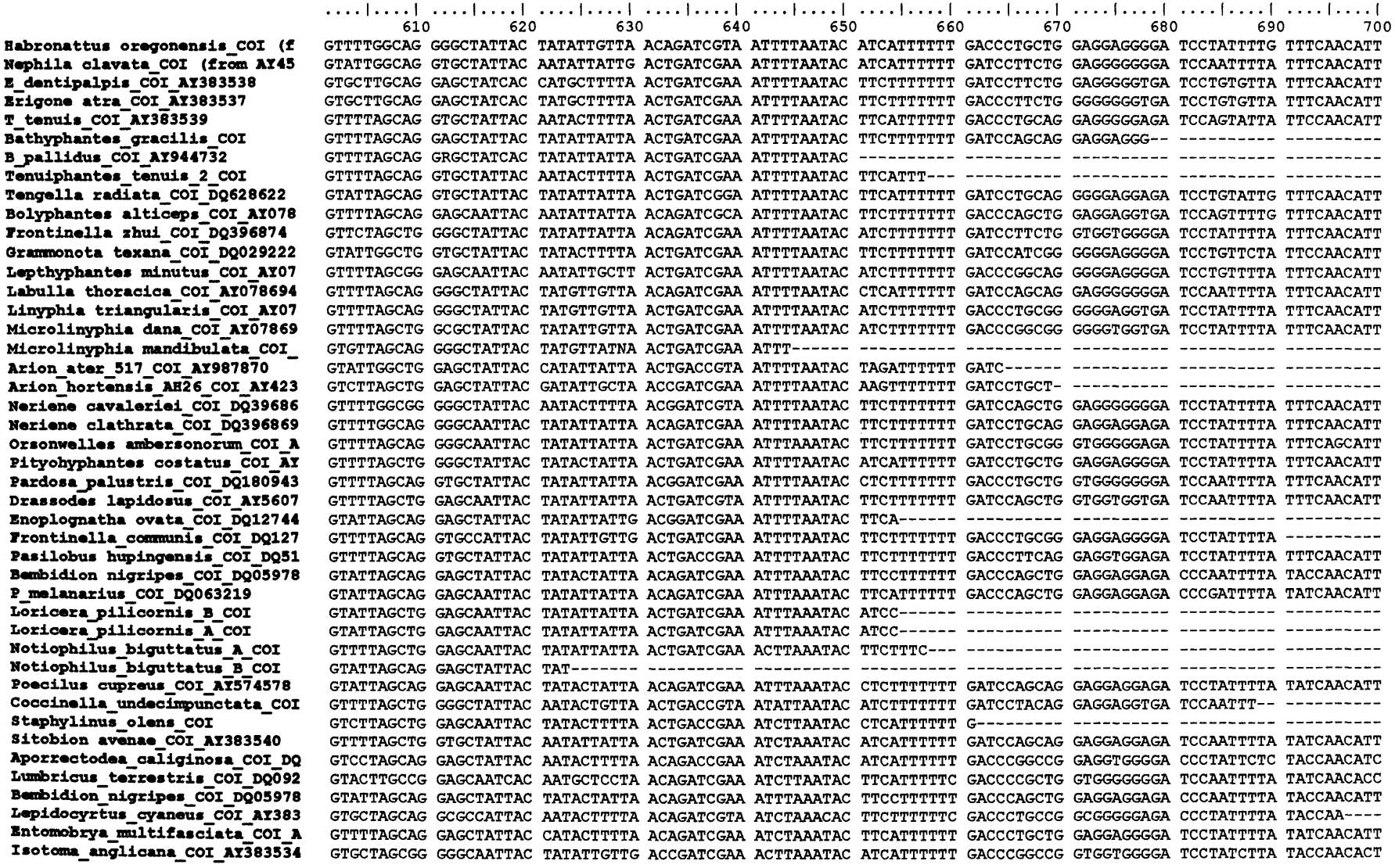
```
                                 ....|....|....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                         110        120        130        140        150        160        170        180        190        200
Habronattus oregonensis_COI (f   GAATATTAAT TCGGATGGAA TTAGGTCAAA CTGGTAGATT TTTGGGGAGT GATCATTTGT ATAATGTTAT CGTTACTGCT CATGCATTTG TTATAATTTT
Nephila clavata_COI (from AY45   GAGTATTAAT TCGTACTGAG CTTGGACAGC CAGGAAGATT TATAGGGGAT GATCAATTAT ATAATGTAAT TGTTACAGCT CATGCATTTG TGATAATTTT
E_dentipalpis_COI_AY383538       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Erigone atra_COI_AY383537        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
T_tenuis_COI_AY383539            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Bathyphantes_gracilis_COI        GAGTTTTAAT TCGAATTGAA TTGGGGCAAA CTGGAAGAAT ATTAGGAGAT GACCATTTAT ATAATGTCAT TGTTACTGCA CATGCTTTTG TTATAATTTT
B_pallidus_COI_AY944732          GAGTTTTAAT TCGAAYTGAG TTGGGTCAAA CGGGAAGAAT GTTAGGGGAT GACCATTTGT ATAATGTAAT TGTAACAGCR CATGCTTTTA TTATAATTTT
Tenuiphantes_tenuis_2_COI        GAGTATTGAT TCGAATTGAG TTGGGTCAAA CTGGGAGAAT ATTGGGAGAT GATCAGTTAT ATAATGTTAT TGTCACTGCA CATGCTTTTG TTATAATTTT
Tengella radiata_COI_DQ628622    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Bolyphantes alticeps_COI_AY078   GAGTATTGAT TCGAATTGAA TTGGGTCAGA CGGGAAGAAT GTTAGGAGAT GATCAATTGT ATAATGTTAT TGTTACAGCA CATGCTTTTG TTATAATTTT
Frontinella zhui_COI_DQ396874    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Grammonota texana_COI_DQ029222   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Lepthyphantes minutus_COI_AY07   GAGTATTAAT TCGAATTGAG TTAGGACAAA CAGGAAGAAT GTTAGGAGAT GATCAAGTGT ATAATGTAAT TGTAACAGCT CATGCGTTTG TAATAATTTT
Labulla thoracica_COI_AY078694   ---------- ---------- ---------- ---------- -------TTT ATAATGTTAT TGTGACAGCC CACGCGTGTG TAATAATTTT
Linyphia triangularis_COI_AY07   GAGTTTTAAT TCGAATTGAA CTTGGACAAA CAGGTAGAAT ACTAGGAGAT GATCAATTGT ATAATGTAAT TGTTACAGCT CATGCCTTTG TAATAATTTT
Microlinyphia dana_COI_AY07869   GAGTTTTAAT TCGAATTGAG CTTGGACAAA CAGGAAGAAT ACTTGGTGAT GACCAATTGT ATAATGTTAT TGTTACTGCC CACGCTTTTG TAATAATTTT
Microlinyphia mandibulata_COI_   GAGTTTTAAT TCGAATTGAG CTTGGTCAGA CAGGGAGAAT GTTGGGGGAT GATCAGTTAT ATAATGTTAT TGTCACTGCT CATGCTTTTG TAATAATTTT
Arion_ater_517_COI_AY987870      CTCTTTTAAT TCGCTTAGAG TTAGGAACAA CTGGTATTTT AACT---GAC GATCAATTTT TTAATGTTAT CATTACCGCA CACGCTTTTG TGATAATTTT
Arion_hortensis_AH26_COI_AY423   CCTTGTTAAT CCGACTTGAG CTCGGTACTA CCGGGGTTTT AACT---GAT GATCAATTTT TTAACGTTAT CGTTACTGCT CACGCTTTTG TAATAATTTT
Neriene cavaleriei_COI_DQ39686   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Neriene clathrata_COI_DQ396869   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Orsonwelles ambersonorum_COI_A   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Pityohyphantes costatus_COI_AY   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Pardosa_palustris_COI_DQ180943   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Drassodes lapidosus_COI_AY5607   GAGTAGTGAT TCGTATGGAA TTAGGACAAT CTGGTAGATT ATTAGGAGAT GATCATTTAT ATAATGTAAT TGTTACAGCA CATGCTTTTG TTATAATTTT
Enoplognatha ovata_COI_DQ12744   GAGCTTTAAT TCGAATTGAG TTGGGGCAAA CTGGGAGATT TTTAGGTGAC GATCAATTAT ATAATGTAAT TGTAACAGCT CATGCATTTG TAATAATTTT
Frontinella_communis_COI_DQ127   GAGTTTTAAT TCGAATTGAG TTAGGGCAAA CTGGAAGATT TTTAGGAGAT GATCAGCTTT ATAATGTTAT TGTAACTGCT CATGCGTTTG TAATAATCTT
Pasilobus hupingensis_COI_DQ51   GAGTATTAAT TCGAATTGAA TTAGGGCAAT CTGGGAGGTT TTTAGGAGAT GATCAGTTAT ATAATGTTAT TGTTACTGCT CATGCCTTTG TTATAATTTT
Bembidion nigripes_COI_DQ05978   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P_melanarius_COI_DQ063219        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Loricera_pilicornis_B_COI        GAATGTTAAT TCGAGCAGAA TTAGGGAATC CAGGTTCTTT TATTGGAGAT GATCAAATTT ATAATGTTAT TGTTACTGCT CATGCATTTG TAATGATTTT
Loricera_pilicornis_A_COI        GAATGTTAAT TCGAGCAGAA TTAGGGAATC CAGGTTCTTT TATTGGAGAT GATCAAATTT ATAATGTTAT TGTTACTGCT CATGCATTTG TAATGATTTT
Notiophilus_biguttatus_A_COI     GAATACTAAT TCGAGCAGAA TTAGGAAATC CTGGATCTTT AATTGGAGAT GACCAAATTT ATAATGTAAT CGTTACAGCT CATGCTTTTG TCATAATTTT
Notiophilus_biguttatus_B_COI     GTATACTAAT TCGAGCAGAA TTAGGAAATC CCGGATCTTT AATTGGTGAT GATCAAATTT ACAATGTAAT TGTAACAGCT CATGCTTTCG TTATAATTTT
Poecilus cupreus_COI_AY574578    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Coccinella_undecimpunctata_COI   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Staphylinus_olens_COI            GTCTACTAAT TCGAGCCGAA CTAGGAAATC CCGGAACATT GATTGGTGAC GATCAAATTT ACAATGTTAT TGTAACAGCT CATGCATTCG TAATGATTTT
Sitobion avenae_COI_AY383540     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Aporrectodea_caliginosa_COI_DQ   GCCTTCTTAT TCGAATTGAA TTAAGGCAAC CGGGGGCATT TCTTGGCAGA GACCAACTCT ATAACACAAT TGTTACTGCC CATGCATTCG TAATAATCTT
Lumbricus_terrestris_COI_DQ092   GACTTCTTAT CCGTATTGAG CTAAGACAAC CTGGTGCATT CCTGGGCAGT GACCAATTAT ATAATACAAT CGTTACTGCA CACGCGTTTG TAATAATCTT
Bembidion_nigripes_COI_DQ05978   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Lepidocyrtus_cyaneus_COI_AY383   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Entomobrya_multifasciata_COI_A   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Isotoma_anglicana_COI_AY383534   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
```

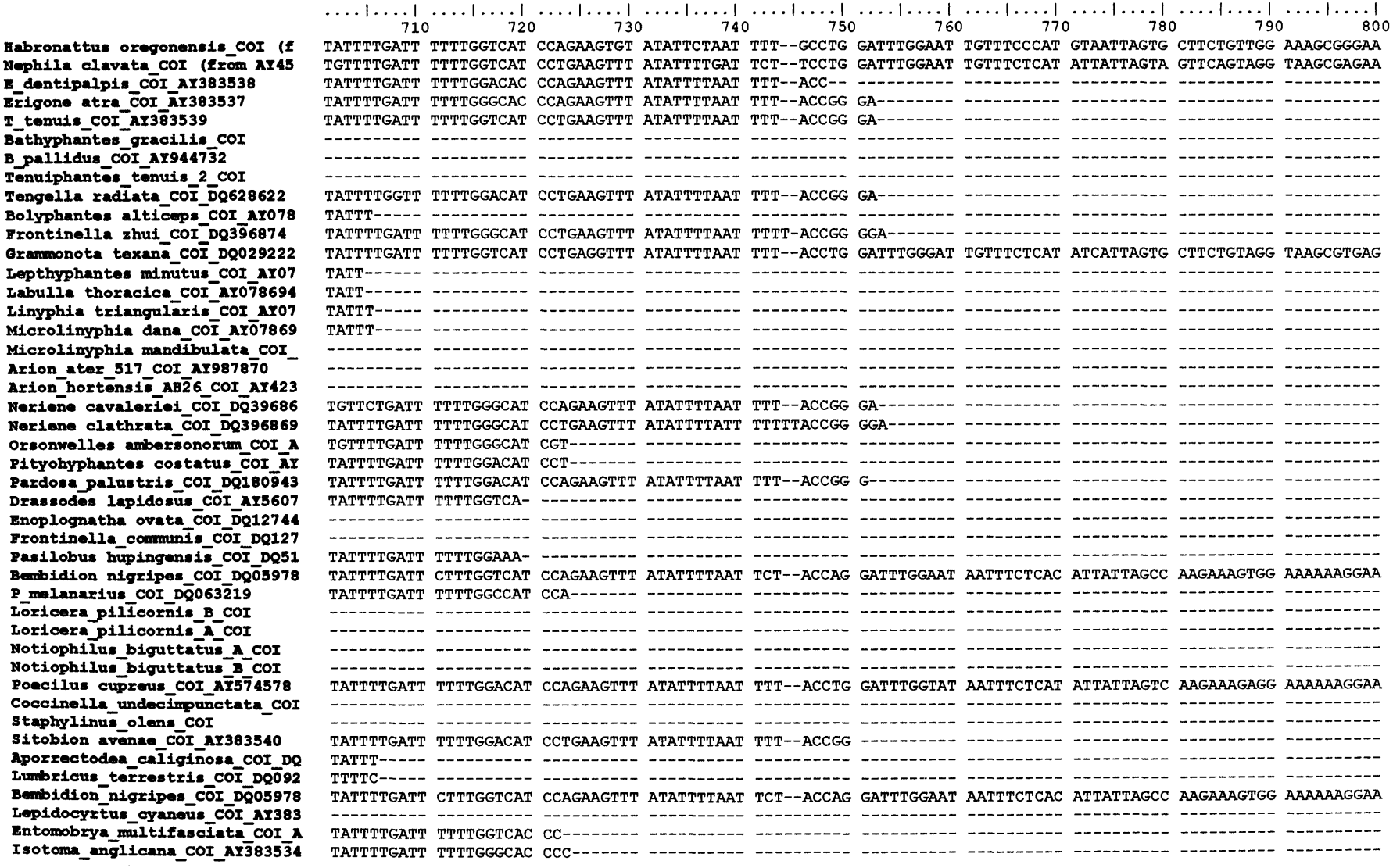

210 220 230 240 250 260 270 280 290 300
Habronattus oregonensis_COI (f TTTTATAGTA ATACCAATTT TAATTGGTGG ATTTGGAAAT TGGTTGGTTC CTTTAATATT GGGGGCTCCT GATATGGCCT TTCCTCGAAT AAATAATTTG
Nephila clavata_COI (from AY45 TTTTATAGTA ATACCAATTT TAATTGGTGG ATTTGGAAGT TGATTGGTTC CTTTAATATT AGGAGCGCCA GATATAGCGT TTCCTCGAAT AAATAATTTA
E_dentipalpis_COI_AY383538 ---------- ---------- ---------- -TTTGGAAAT TGATTAGTTC CTTTAATATT AGGGGCTCCT GATATAGCTT TTCCTCGTAT AAATAATTTA
Erigone atra_COI_AY383537 ---------- ---------- ----TGGAGG AATTGGAAAT TGATTAGTTC CTTTAATATT AGGGGCTCCT GATATAGCTT TTCCTCGTAT AAATAATTTA
T_tenuis_COI_AY383539 ---------- ---------- -----GGAGG ATTTGGAAAT TGATTAGTTC CTTTGATACT TGGGGCACCT GATATAGCTT TTCCTCGAAT AAATAATTTA
Bathyphantes_gracilis_COI TTTTATAGTG ATACCTATTT TGATTGGGGG GTTTGGTAAT TGGTTGGTTC CTTTAATATT AGGGGCTCCA GATATAGCTT TTCCTCGAAT GAATAATTTA
B_pallidus_COI_AY944732 TTTTATAGTT ATACCTATTT TAATTGGGGG TTTTGGTAAT TGATTAGTRC CTTTAATATT AGGGGCTCCG GATATAGCTT TTCCTCGTAT AAATAATTTA
Tenuiphantes_tenuis_2_COI TTTTATAGTT ATACCCATTT TAATTGGGGG ATTTGGAAAT TGGTTAGTTC CTTTGATACT TGGGGCACCT GATATAGCTT TTCCTCGAAT AAATAATTTA
Tengella radiata_COI_DQ628622 ---------- ---------- ---------- GTTTGGAAAT TGATTAGTAC CTTTAATATT AGGGGCACCA GATATAGCTT TTCCTCGGAT AAATAATTTA
Bolyphantes alticeps_COI_AY078 TTTTATAGTA ATGCCAATTT TAATTGGAGG GTTTGGAAAT TGATTGGTTC CTTTAATGTT GGGGGCTCCT GATATAGCTT TTCCTCGAAT AAATAACTTG
Frontinella zhui_COI_DQ396874 ---------- ---------- ---------- ---------- ---------- ---------- ---------- GATATAGCTT TTCCTCGAAT AAATAATTTA
Grammonota texana_COI_DQ029222 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----TCGGAT AAATAATTTG
Lepthyphantes minutus_COI_AY07 TTTTATAGTT ATACCAATTT TGATTGGAGG ATTTGGAAAC TGGTTAGTAC CTTTAATACT TGGGGCACCG GATATGGCTT TTCCTCGTAT GAATAATTTA
Labulla thoracica_COI_AY078694 TTATAGGGAT ACGCCTATTT TAATTGGGGG ATTTGGAAAT TGGTTAATTC CTTTAATGTT AGGGGCGCCT GATATGGCTT TTCCTCGAAT AAATAATTTG
Linyphia triangularis_COI_AY07 TTTTATAGTT ATACCAATTT TAATCGGGGG GTTTGGTAAT TGGCTAGTAC CTTTAATGTT GGGGGCTCCT GATATAGCTT TTCCTCGGAT GAATAACTTA
Microlinyphia dana_COI_AY07869 TTTTATGGTT ATACCTATTT TAATTGGGGG GTTTGGAAAT TGGTTAGTCC CTTTAATGTT AGGGGCTCCT GATATAGCAT TTCCTCGAAT AAATAATTTG
Microlinyphia mandibulata_COI_ TTTTATAGTA ATGCCTATTT TGATTGGTGG GTTTGGTAAT TGATTAGTTC CTTTAATGTT AGGAGCTCCA GATATGGCAT TTCCTCGAAT AAATAATTTA
Arion_ater_517_COI_AY987870 TTTTATGGTT ATGCCTTTAA TAATTGGGGG GTTTGGTAAT TGAATAGTGC CCTTACTTAT CGGGGCCCCC GATATGAGAT TTCCTCGTAT AAATAATATA
Arion_hortensis_AH26_COI_AY423 TTTTATGGTT ATACCCTTAA TGATCGGGGG CTTTGGAAAT TGAATAGTGC CACTATTATT AGGGGCTCCG GACATAAGGT TCCCTCGAAT GAATAATATA
Neriene cavaleriei_COI_DQ39686 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Neriene clathrata_COI_DQ396869 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---ATAGCTT TTCCTCGTAT AAATAATTTG
Orsonwelles ambersonorum_COI_A ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----TCGAAT GAATAATTTA
Pityohyphantes costatus_COI_AY ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----TCGAAT AAATAATTTA
Pardosa_palustris_COI_DQ180943 ---------- ---------- -----GGAGG ATTTGGAAAT TGATTAGTTC CTTTAATATT AGGTGCTCCT GATATAGCAT TCCCACGAAT AAATAATCTT
Drassodes lapidosus_COI_AY5607 TTTTATAGTA ATACCTATTT TGATTGGAGG CTTTGGAAAT TGATTAGTTC CTTTAATATT AGGTGCTCCT GATATAGCTT TTCCACGTAT GAATAATTTA
Enoplognatha ovata_COI_DQ12744 TTTTATAGTT ATACCTATTT TAATTGGAGG TTTTGGGAAT TGGTTAGTTC CTTTAATATT AGGAGCTCCT GATATGGCTT TTCCTCGAAT AAATAATTTA
Frontinella_communis_COI_DQ127 TTTTATAGTT ATACCAATTT TAATTGGAGG ATTTGGTAAT TGGTTAGTTC CTCTTATGTT AGGGGCTCCT GATATAGCTT TTCCTCGAAT GAATAATTTA
Pasilobus hupingensis_COI_DQ51 TTTTATAGTA ATACCTATTT TGATTGGGGG TTTTGGAAAT TGATTAGTTC CATTAATGTT AGGGGCTCCT GATATAGCTT TTCCTCGAAT AAATAATTTA
Bembidion nigripes_COI_DQ05978 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----TCGAAT AAATAATATA
P_melanarius_COI_DQ063219 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----CGAAT AAATAATATA
Loricera_pilicornis_B_COI TTTTATAGTT ATACCAATTA TAATTGGAGG ATTTGGAAAC TGACTTGTTC CTTTAATATT AGGTGCCCCT GATATAGCCT TTCCTCGAAT AAATAATATA
Loricera_pilicornis_A_COI TTTTATAGTT ATACCAATTA TAATTGGAGG ATTTGGAAAC TGGCTTGTTC CTTTAATATT AGGTGCCCCT GATATAGCCT TTCCTCGAAT AAATAATATA
Notiophilus_biguttatus_A_COI TTTTATAGTT ATACCTATTA TAATTGGAGG ATTTGGTAAT TGACTTGTAC CTCTAATATT AGGAGCTCCT GATATAGCTT TTCCTCGAAT AAATAATATG
Notiophilus_biguttatus_B_COI TTTTATAGTA ATGCCTATTA TAATTGGGGG GTTTGGTAAT TGATTAGTTC CTCTAATGTT AGGGGCTCCT GATATGGCCT TTCCTCGAAT AAATAATATA
Poecilus cupreus_COI_AY574578 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----TCGAAT AAATAATATA
Coccinella_undecimpunctata_COI ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----ACGTTT AAATAATATA
Staphylinus_olens_COI CTTCATAGTT ATACCTGTTG TAATTGGTGG ATTTGGAAAT TGATTAGTCC CACTAATACT CGGTGCACCT GACATAGCTT TTCCTCGAAT AAACAACATA
Sitobion avenae_COI_AY383540 ---------- ---------- ----TGGAGG AATTGGAAAT TGATTAGTTC CTATAATAAT AGGATGTCCT GACATATCAT TCCCACGTTT AAACAATATT
Aporrectodea_caliginosa_COI_DQ CTTCTTAGTT ATACCAGTAT TTATTGGGGG GTTTGGAAAT TGACTACTAC CATTAATACT GGGCGCTCCC GACATAGCAT TTCCACGACT AAATAACATA
Lumbricus_terrestris_COI_DQ092 CTTTCTGGTA ATACCAGTCT TCATCGGCGG GTTTGGAAAC TGACTTCTTC CCCTAATACT AGGCGCCCCT GATATAGCAT TCCCACGCCT TAATAACATA
Bembidion_nigripes_COI_DQ05978 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----TCGAAT AAATAATATA
Lepidocyrtus_cyaneus_COI_AY383 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- TTCCACGAAT AAACAACATA
Entomobrya_multifasciata_COI_A ---------- ---------- ---------- ----GGAAAT TGATTAGTTC CTCTAATAAT TGGTGCCCCA GATATAGCTT TTCCTCGAAT AAACAACATA
Isotoma_anglicana_COI_AY383534 ---------- ---------- ---------- -TTTGGAAAT TGATTAGTTC CTTTAATAAT TGGAGCGCCG GATATGGCCT TCCCCCGAAT AAATAATATA


310 320 330 340 350 360 370 380 390 400
Habronattus oregonensis_COI (f AGTTTTTGAT TATTACCTCC TTCTTTAATA TTATTATTTA TTTCCTCTAT AGTAGAGATA GGAGTTGGTG CAGGGTGAAC AGTATATCCT CCATTAGCAT
Nephila clavata_COI (from AY45 AGGTTTTGAT TATTGCCTCC TTCTTTATTT TTATTGTTCG TTTCTTCAAT AGTAGAGATA GGAGTGGGAG CAGGTTGGAC AGTTTACCCT CCTTTATCAT
E_dentipalpis_COI_AY383538 AGATTTTGAT TATTACCCCC TTCTTTATTA TTATTATTTA TCTCTAGAAT AGATGAAATA GGTGTAGGGG CGGGATGAAC AATTTATCCT CCTCTAGCTT
Erigone atra_COI_AY383537 AGATTTTGAT TATTACCTCC TTCTTTATTA TTATTATTTA TCTCTAGTAT AGATGAGATA GGTGTGGGGG CGGGATGAAC AATTTATCCT CCTCTAGCTT
T_tenuis_COI_AY383539 AGCTTTTGAC TTTTACCCCC TTCTTTGTTA TTACTATTTA TTTCAAGTAT GGTTGAGATA GGAGTTGGGG CAGGGTGAAC AGTGTATCCT CCTCTTGCTT
Bathyphantes_gracilis_COI AGATTTTGGT TACTCCCTCC TTCATTATTA ATATTATTTA TTTCTTCAAT AGTAGAAATA GGAGTTGGAG CAGGTTGAAC TGTTTATCCT CCTTTAGCTT
B_pallidus_COI_AY944732 AGTTTTTGGT TACTTCCTCC TTCTTTATTA ATATTATTTA TTTCTTCTAT AGTAGAGATR GGTGTTGGAG CTGGTTGGAC TGTATATCCG CCATTGGCTG
Tenuiphantes_tenuis_2_COI AGCTTTTGAC TTTTACCCCC TTCTTTGTTA TTACTATTTA TTTCAAGTAT GGTTGAGATA GGAGTTGGGG CAGGGTGAAC AGTGTATCCT CCTCTTGCTT
Tengella radiata_COI_DQ628622 TCTTTTTGAT TATTACCTCC TTCTTTAATG TTGTTATTAG TGTCTTCTAT AGTAGAGTTA GGTGTTGGTG CTGGATGGAC GGTTTATCCT CCTTTAGCTT
Bolyphantes alticeps_COI_AY078 AGTTTTTGGT TATTACCCCC TTCTCTACTT TTATTATTTA TTTCAAGTAT GGTTGAGATA GGGGTTGGGG CTGGATGAAC TGTGTATCCT CCACTTGCTT
Frontinella zhui_COI_DQ396874 AGATTTTGAT TATTACCTCC TTCTTTATTT TTTTTGTTTA TTTCCTCAAT AGTAGAGGTT GGGGTAGGGG CTGGTTGGAC TGTATATCCC CCATTATCTT
Grammonota texana_COI_DQ029222 AGATTTTGAT TGTTGCCTCC TTCTTTATTG TTGTTATTTA TTTCTAGTAT GGATGAAATG GGAGTGGGGG CAGGTTGAAC TATTTATCCT CCTCTTGCTT
Lepthyphantes minutus_COI_AY07 AGTTTTTGAC TTCTACCCCC TTCCCTTTTA TTATTATTTA TTTCAAGAAT AGTAGAAATA GGAGTAGGTG CTGGGTGAAC GGTTTATCCC CCTCTTGCTT
Labulla thoracica_COI_AY078694 AGTTTTTGAT TATTACCTCC TTCTTTAATG TTATTATTTA TTTCTTCTAT GGTAGAAATA GGTGTTGGGG CTGGTTGGAC TGTGTATCCG CCATTGGCTT
Linyphia triangularis_COI_AY07 AGTTTTTGAT TATTACCTCC TTCTTTATTC TTATTATTTA TTTCTTCTAT GGCTGAAGTA GGTGTGGGAG CTGGGTGAAC AGTTTATCCT CCTTTGTCTT
Microlinyphia dana_COI_AY07869 AGTTTTTGAT TATTACCCCC TTCTTTGTTA TTATTATTTA TTTCTTCTAT GGTTGAGATA GGTGTTGGGT CAGGGTGAAC TGTTTATCCC CCCTTGTCTT
Microlinyphia mandibulata_COI_ AGTTTTTGGT TGTTACCTCC TTCTCTGTTA TTATTATTTA TTTCTTCAAT AGTTGAAATG GGTGTTGGGG CTGGATGAAC TGTTTATCCT CCTTTATCTT
Arion_ater_517_COI_AY987870 AGATTTTGAT TATTACCGCC ATCTTTACTT TTACTTATTT GTTCTAGTAT AGTAGAAGGA GGAGCTGGAA CAGGTTGAAC AGTGTACCCA CCACTGAGAG
Arion_hortensis_AH26_COI_AY423 AGTTTCTGAC TTCTCCCCCC TTCTCTACTT TTACTTATTG TATCAAGTAT GGTGGAAGGT GGGGCCGGCA CGGGTTGGAC AGTTTATCCT CCCTTAAGAA
Neriene cavaleriei_COI_DQ39686 -------GAC TTTTACCTCC TTCTTTATTT TTATTATTTA TATCTTCAAT AGTTGAAATA GGGGTAGGAG CAGGATGAAC TGTGTACCCT CCATTATCCT
Neriene clathrata_COI_DQ396869 AGTTTTTGAC TTTTGCCTCC TTCTTTATTT TTGTTATTTA TCTCTTCTAT AGTGGAAATA GGAGTAGGAG CAGGTTGAAC GGTTTATCCA CCTCTTTCTT
Orsonwelles ambersonorum_COI_A AGATTTTGAT TATTACCACC TTCTCTATTA TTATTATTTA TTTCCTCTAT GGTAGAAATA GGAGTAGGAG CAGGATGAAC AGTGTATCCT CCTTTATCTT
Pityohyphantes costatus_COI_AY AGTTTTTGAT TACTTCCTCC TTCATTATTA TTGTTATTTA TTTCATCTAT AGTAGAAATA GGAGTTGGGG CAGGTTGAAC TGTTTATCCT CCTTTATCTT
Pardosa_palustris_COI_DQ180943 TCTTTTTGAT TATTACCTCC TTCTTTATTT TTATTATCTA TATCTTCTAT AGTGGAGATA GGAGTTGGTG CTGGATGAAC TGTTTATCCT CCTTTAGCAT
Drassodes lapidosus_COI_AY5607 AGTTTTTGAT TATTACCACC TTCATTAGTT TTATTATTTA TTTCTTCTAT AGCTGAGATA GGTGTAGGAG CTGGTTGAAC AGTTTATCCT CCTTTGGCTT
Enoplognatha ovata_COI_DQ12744 AGTTTTTGAT TACTACCTCC TTCATTAATA TTATTATTTG TTTCTTCAAT AGTAGAAATA GGAGTAGGAG CTGGGTGGAC AGTTTACCCT CCTTTATCTT
Frontinella_communis_COI_DQ127 AGTTTTTGGT TACTTCCCCC TTCTTTATTT TTGTTATTTA TTTCTTCAAT ATCAGAAATA GGGGTTGGAG CTGGGTGAAC TGTGTATCCT CCTTTATCTT
Pasilobus hupingensis_COI_DQ51 AGTTTTTGAT TGTTACCCCC ATCCTTATTT CTTTTAATTA TTTCATCAAT AGTAGAGATA GGGGTGGGAG CTGGTTGAAC AGTATACCCG CCTTTGGCGA
Bembidion nigripes_COI_DQ05978 AGTTTTTGAC TTCTTCCGCC TTCATTAACT CTTTTATTAA TGAGAAGATT AGTAGAAAGT GGAGCAGGTA CTGGATGAAC AGTTTACCCT CCTTTATCAT
P_melanarius_COI_DQ063219 AGTTTTTGAC TTCTTCCTCC TTCATTAACT CTTCTTTTAA TGAGAAGTAT AGTTGAAAGT GGAGCTGGTA CTGGATGAAC AGTTTACCCC CCTTTATCTT
Loricera_pilicornis_B_COI AGATTTTGAC TCCTTCCACC ATCTCTTACT CTTCTCCTAC TTAGAAGAAT GGTAGAGAGA GGTGCTGGTA CAGGATGGAC CGTGTACCCT CCTCTTTCTT
Loricera_pilicornis_A_COI AGATTTTGAC TCCTTCCACC ATCTCTTACT CTTCTCCTAC TTAGAAGAAT GGTGGAGAGA GGTGCTGGTA CAGGATGGAC CGTGTACCCT CCTCTTTCTT
Notiophilus_biguttatus_A_COI AGTTTCTGGT TACTTCCTCC CTCTTTAACT CTCCTCCTTA CAAGAAGAAT AGTGGAAAGA GGAGCAGGTA CAGGTTGAAC TGTGTACCCT CCCCTTTCTT
Notiophilus_biguttatus_B_COI AGTTTTTGGT TATTACCACC TTCTTTAACT CTCCTTCTTA CAAGCAGAAT AGTAGAAAGA GGAGCGGGAA CTGGTTGAAC TGTCTACCCT CCCCTTTCTT
Poecilus cupreus_COI_AY574578 AGTTTTTGAC TACTTCCCCC TTCTTTAACT CTTCTTTTAA TGAGAAGTAT GGTAGAAAGT GGAGCAGGAA CAGGATGAAC AGTTTATCCT CCCCTATCTT
Coccinella_undecimpunctata_COI AGATTCTGAT TATTACCTCC TGCCTTAACT CTCTTAATCA TTAGAAGATT AGTAGAAATA GGAGCAGGAA CAGGTTGAAC TGTTTACCCA CCTTTATCTT
Staphylinus_olens_COI AGGTTTTGAC TTCTCCCTCC GTCATTAACT TTACTGCTTA TGAGAAGAAT AGCTGAAAGA GGTGCAGGAA CAGGATGAAC TGTGTATCCT CCACTTTCCA
Sitobion avenae_COI_AY383540 AGATTTTGAT TACTACCACC ATCATTAATA ATAATAATCT GTAGTTTCTT AATCAATAAT GGAACAGGTA CAGGATGAAC TATTTACCCA CCTTTATCAA
Aporrectodea_caliginosa_COI_DQ AGATTCTGAT TACTTCCCCC CTCTTTAATC CTTCTAGTTT CATCTGCTGC CGTTGAAAAG GGGGCTGGCA CAGGATGAAC TGTATATCCT CCACTATCAA
Lumbricus_terrestris_COI_DQ092 AGATTCTGAC TTCTACCCCC CTCTCTCATT CTCCTAGTTT CCTCAGCTGC CGTAGAGAAA GGAGCCGGAA CAGGGTGAAC AGTATACCCC CCTCTTGCCA
Bembidion_nigripes_COI_DQ05978 AGTTTTTGAC TTCTTCCGCC TTCATTAACT CTTTTATTAA TGAGAAGATT AGTAGAAAGT GGAGCAGGTA CTGGATGAAC AGTTTACCCT CCTTTATCAT
Lepidocyrtus_cyaneus_COI_AY383 AGTTTCTGAC TTTTACCTCC ATCACTAACC CTTTTATTAG CTGGAGGTCT AGTAGAAAGA GGTGCCGGCA CAGGATGAAC AGTATACCCC CCACTAGCTG
Entomobrya_multifasciata_COI_A AGATTCTGAC TCCTACCCCC CTCTCTAACC CTCCTTCTTA CAGGAGGTTT AGTAGAAAGA GGAGCCGGAA CTGGTTGAAC AGTTTACCCC CCATTAGCTG
Isotoma_anglicana_COI_AY383534 AGATTTTGAC TTCTTCCCCC GTCTTTAACT CTTCTATTGG CCGGAGGACT TGTTGAAAGA GGAGCAGGAA CAGGATGAAC AGTGTACCCT CCGCTTTCTT


410 420 430 440 450 460 470 480 490 500
Habronattus oregonensis_COI (f CTGTGGTTGG ACATGGAGGA AGTTCGGTGG ATTTTGCTAT TTTTTCATTA CATTTAGCAG GTGCTTCTTC TATTATAGGA GCTATTAATT TTATTTCTAC
Nephila clavata_COI (from AY45 CTTTAGATGG ACATTCTGGA AGGTCAGTGG ATTTTGCTAT TTTTTCTTTA CATTTAGCTG GGGCTTCTTC AATTATAGGG GCTATTAATT TTATTTCTAC
E_dentipalpis_COI_AY383538 CTTTAGAGGG TCATTCTGGT AGTTCTGTTG ATTTTGCAAT TTTCTCTTTA CACTTAGCTG GTGCTTCTTC TATTATAGGG GCTATTAATT TTATTTCTAC
Erigone atra_COI_AY383537 CTTTAGAGGG TCATTCTGGT AGTTCTGTTG ATTTTGCAAT TTTTTCTTTA CACTTAGCTG GTGCTTCTTC TATTATAGGG GCTATTAATT TTATTTCTAC
T_tenuis_COI_AY383539 CTTTGGAAGG TCATGCAGGA AGTTCTGTGG ACTTTGCTAT TTTTTCTCTT CATTTAGCTG GGGCTTCTTC TATTATAGGA GCAATTAATT TTATTTCTAC
Bathyphantes_gracilis_COI CTTTAGAAGG GCATTCAGGG AGATCTGTGG ATTTTGCTAT TTTTTCTTTA CATTTAGCTG GGGCTTCGTC AATTATAGGG GCTATTAATT TTATTTCGAC
B_pallidus_COI_AY944732 GATTGGAAGG GCATTCTGGG AGATCTGTGG ATTTTGCCAT TTTTTCATTA CATTTAGCAG GGGCTTCTTC TATTATGGGR GCTATTAATT TTATTTCTAC
Tenuiphantes_tenuis_2_COI CTTTGGAAGG TCATGCAGGA AGTTCTGTGG ACTTTGCTAT TTTTTCTCTT CATTTAGCTG GGGCTTCTTC TATTATAGGA GCAATTAATT TTATTTCTAC
Tengella radiata_COI_DQ628622 CT---GAGGG TCATATGGGA AGAGCTGTTG ATTTTGCAAT TTTTTCTTTA CATTTAGCTG GGGCTTCATC TATTATAGGG GCTATTAATT TTATTTCTAC
Bolyphantes alticeps_COI_AY078 CTTTAGAGGG ACATGCTGGT AGTTCTGTTG ATTTTGCTAT TTTTTCTCTT CATTTAGCCG GGGCTTCTTC AATTATAGGT GCTATTAATT TTATCTCGAC
Frontinella zhui_COI_DQ396874 CTTTGGAGGG GCATTCGGGT AGATCTGTTG ATTTTGCTAT TTTTTCTTTA CATTTAGCAG GAGCTTCATC TATTATAGGA GCTATTAATT TTATTTCTAC
Grammonota texana_COI_DQ029222 CTTTAGAAGG GCATTCTGGG AGTTCGGTAG ATTTTGCTAT TTTTTCTTTA CATTTGGCAG GGGCTTCTTC TATTATAGGA GCTATTAATT TTATTTCTAC
Lepthyphantes minutus_COI_AY07 CTTTAGAGGG GCATGCTGGA AGATCTGTAG ATTTTGCTAT TTTTTCTCTT CATTTAGCAG GAGCTTCTTC TATTATAGGG GCAATTAATT TTATTTCTAC
Labulla thoracica_COI_AY078694 CTTTGGAAGG GCATGCTGGA AGTTCAGTAG ATTTTGCTAT TTTTTCTTTA CATTTAGCGG GGGCTTCTTC TATTATAGGG GCTATTAATT TTATTTCTAC
Linyphia triangularis_COI_AY07 CTTTGGAGGG TCATTCAGGG AGTTCTGTTG ATTTTGCTAT TTTTTCTTTA CATTTAGCTG GAGCATCTTC AATTATGGGG GCTATTAATT TTATTTCTAC
Microlinyphia dana_COI_AY07869 CGTTAGAAGG CCATTCGGGG AGATCTGTTG ATTTTGCTAT TTTTTCTCTT CATTTAGCTG GTGCTTCTTC AATTATAGGA GCTATTAATT TTATTTCGAC
Microlinyphia mandibulata_COI_ CTTTGGAAGG GCATTCAGGA AGTTCTGTTG ATTTTGCAAT CTTTTCTCTA CATTTAGCTG GAGCTTCTTC AATTATGGGA GCTATTAATT TTATTTCTAC
Arion_ater_517_COI_AY987870 GAGCCTTAGC ACATAGCGGA GCTTCAGTTG ACTTAGCAAT TTTTTCGTTA CATTTAGCTG GTATATCTTC TATTTTAGGA GCAATTAATT TTATTTCTAC
Arion_hortensis_AH26_COI_AY423 GAATTATAGC TCATAGGGGG GCATCTGTAG ACTTAGCCAT CTTTTCCTTA CATTTAGCGG GAATGTCTTC GATTTTGGGT GCTATTAATT TTATTACGAC
Neriene cavaleriei_COI_DQ39686 CTCTTGAAGG ACATTCAGGT GTGTCTATAG ATTTTGCTAT TTTTTCTTTA CATTTAGCTG GGGCTTCTTC TATTATAGGG GCTATTAACT TTATTTCTAC
Neriene clathrata_COI_DQ396869 CATTAGAGGG ACATTCTGGA AGATCTGTGG ATTTTGCTAT TTTTTCTTTA CATTTAGCAG GAGCTTCTTC TATTATGGGG GCAATTAATT TTATTTCTAC
Orsonwelles ambersonorum_COI_A CTTTAGAAGG TCATTCAGGG AGATCAGTTG ATTTTGCTAT TTTTTCTTTG CATTTGGCTG GGGCTTCGTC AATTATAGGA GCTATTAATT TTATTTCTAC
Pityohyphantes costatus_COI_AY CATTAGAAGG ACATTCTGGG GGATCTGTTG ATTTTGCTAT TTTTTCTTTA CATTTAGCAG GGGCTTCTTC TATTATAGGG GCTATTAATT TTATTTCCAC
Pardosa_palustris_COI_DQ180943 CTACAGTTGG TCATATAGGA AGTTCAATAG ATTTTGCTAT TTTTTCTCTT CATTTAGCTG GTGCTTCTTC TATTATAGGG GCTGTTAATT TTATTTCTAC
Drassodes lapidosus_COI_AY5607 CTAATGTTGG ACATGCTGGA AGTGCTATGG ATTTTGCTAT TTTTTCATTA CATTTGGCTG GAGCTTCTTC TATTATAGGA GCTGTTAATT TTATTTCTAC
Enoplognatha ovata_COI_DQ12744 CTTTGGAAGG ACATGCTGGT AGATCTGTAG ATTTTGCTAT TTTTTCTTTA CATTTAGCTG GTGCTTCATC TATTATAGGA GCTATTAATT TTATTTCTAC
Frontinella_communis_COI_DQ127 CTTTAAAAGG CCATTCAGGA AGATCTGTTG ATTTTGCGAT TTTTTCTTTA CATTTGGCTG GGGCTTCATC TATTATAGGG GCTATTAATT TTATTTCTAC
Pasilobus hupingensis_COI_DQ51 GATTAGAGGG TCATGCTGGG AGATCAGTAG ATTTTGCAAT TTTTTCTTTA CATTTAGCTG GGGCTTCTTC AATTATAGGG GCTATCAATT TTATTTCAAC
Bembidion nigripes_COI_DQ05978 CTGGAATTGC ACATAGAGGA GCTTCAGTTG ATCTAGCTAT TTTTAGCCTA CATTTAGCCG GAGTATCCTC TATTTTAGGA GCAGTAAATT TTATTACAAC
P_melanarius_COI_DQ063219 CAGGAATTGC CCATGCTGGA GCTTCAGTTG ATTTGGCTAT TTTTAGACTA CATTTAGCCG GAGTATCATC TATTTTAGGA GCTGTAAATT TTATTACTAC
Loricera_pilicornis_B_COI CTGTTATTGC TCATAGAGGG GCTTCAGTAG ATTTAGCTAT TTTTAGTCTT CACTTAGCAG GAGTATCATC AATTTTAGGA GCTGTAAATT TTATTAGTAC
Loricera_pilicornis_A_COI CTGTTATTGC TCATAGAGGG GCTTCAGTAG ATTTAGCTAT TTTTAGTCTT CACTTAGCAG GAGTATCATC AATTTTAGGA GCTGTAAATT TTATTAGTAC
Notiophilus_biguttatus_A_COI CTGGAATTGC CCATAGAGGA GCTTCAGTTG ATTTAGCAAT TTTTAGCCTT CATTTAGCTG GAGTATCTTC TATTTTAGGA GCAGTAAATT TTATTACTAC
Notiophilus_biguttatus_B_COI CTGGTATTGC CCATAGAGGA GCTTCTGTTG ATTTAGCTAT TTTTAGTCTA CATTTAGCAG GAGTATCTTC TATTTTAGGA GCTGTAAATT TTATTACTAC
Poecilus cupreus_COI_AY574578 CTGGTATTGC CCATGCTGGA GCATCAGTTG ATTTAGCTAT TTTTAGTTTA CATTTAGCAG GAGTTTCTTC AATTTTAGGA GCAGTAAATT TTATTACTAC
Coccinella_undecimpunctata_COI CTAATTTAGC TCATAATGGA CCTTCTGTAG ATTTAGTAAT TTTTAGATTA CACTTAGCAG GAATTTCTTC AATTCTTGGA GCTGTAAATT TCATCTCTAC
Staphylinus_olens_COI CCAATATTGC CCATAGAGGT GCTTCTGTTG ATTTGGCTAT TTTCAGATTA CATTTAGCTG GAATCTCATC AATTTTGGGT GCTGTAAATT TCATTACTTC
Sitobion avenae_COI_AY383540 ATAATATTGC ACATAATAAT ATTTCAGTCG ATTTAACTAT TTTTTCATTA CATTTAGCAG GAATCTCATC AATTTTAGGA GCAATTAATT TTATTTGTAC
Aporrectodea_caliginosa_COI_DQ GAAATCTGGC TCATGCTGGA CCCTCTGTTG ACTTAGCCAT TTTCTCACTC CACTTAGCAG GGGCATCATC AATTCTGGGG GCCATCAACT TTATTACAAC
Lumbricus_terrestris_COI_DQ092 GAAATCTCGC CCATGCCGGG CCATCTGTAG ATTTAGCTAT TTTTTCCCTC CATTTAGCAG GTGCATCCTC TATTCTAGGA GCTATTAATT TTATTACTAC
Bembidion_nigripes_COI_DQ05978 CTGGAATTGC ACATAGAGGA GCTTCAGTTG ATCTAGCTAT TTTTAGCCTA CATTTAGCCG GAGTATCCTC TATTTTAGGA GCAGTAAATT TTATTACAAC
Lepidocyrtus_cyaneus_COI_AY383 CTGGAATCGC CCATGCCGGA GCATCAGTAG ATCTTTCTAT TTTTAGCCTT CATTTAGCTG GGGCTTCTTC AATTTTAGGA GCTGTAAACT TCATCACCAC
Entomobrya_multifasciata_COI_A CAGGTATTGC TCACGCTGGA GCTTCTGTTG ACCTATCTAT TTTCAGTTTA CATTTAGCAG GAGCCTCATC AATTTTAGGA GCAGTAAATT TTATCACAAC
Isotoma_anglicana_COI_AY383534 CAGGTATCGC GCATGCTGGA GCATCTGTGG ACTTATCTAT TTTTAGTTTA CATTTAGCAG GAGCGTCTTC TATTTTAGGG GCTGTAAACT TTATTACAAC


510 520 530 540 550 560 570 580 590 600
Habronattus oregonensis_COI (f TGTAATTAAT ATACGTTCTT CTAGAATATC AATAGATAGG GTTCCTTTAT TTGTATGATC AGTTTTAATT ACAGCTGTGT TGTTATTACT TTCTTTACCT
Nephila clavata_COI (from AY45 TATTGTGAAT ATACGATCTT TTGGAATAAC TATAGAAAAG GTACCTTTAT TTGTTTGGTC TGTTTTAATT ACTGCTGTTT TATTACTTTT ATCTTTACCT
E_dentipalpis_COI_AY383538 AATTTTAAAT ATGCGTGGGT ATGGGATAAC TATAGAAAAA GTTCCTTTAT TTGTATGGTC TGTTTTAATT ACAGCCGTAT TGTTATTATT ATCTTTACCT
Erigone atra_COI_AY383537 AATTTTAAAT ATGCGTGGGT ATGGAATAAC TATAGAAAAA GTTCCTTTAT TTGTATGGTC TGTTTTAATT ACAGCTGTAC TGTTATTATT ATCTTTACCT
T_tenuis_COI_AY383539 TATTTTGAAT ATACGAGGGT ATGGGGTATC TATAGAAAAA GTACCATTGT TTGTTTGATC CGTGTTAATT ACTGCTGTTC TCTTATTATT ATCATTACCT
Bathyphantes_gracilis_COI AATTATAAAT ATACGAGCTT ATGATATCAG AATAGAAAAG GTTTCTTTAT TTGTTTGATC AGTATTGATT ACTGCAGTTT TATTATTATT ATCTTTACCT
B_pallidus_COI_AY944732 AATTTTAAAT ATACGAGCGT ATAATATCAG AATGGAGAAG GTTTCTTTAT TTGTATGATC AGTATTGATT ACGGCTGTTC TTTTATTRTT ATCTCTACCT
Tenuiphantes_tenuis_2_COI TATTTTGAAT ATACGAGGGT ATGGGGTATC TATAGAAAAA GTACCATTGT TTGTTTGATC CGTGTTAATT ACTGCTGTTC TCTTATTATT ATCATTACCT
Tengella radiata_COI_DQ628622 TATTATTAAT ATACGATGTT ATGGGGTAAG AATGGAGAAA GTTTCATTAT TTGTTTGATC AGTATTAATT ACTGCTGTTT TATTGTTGTT ATCATTGCCT
Bolyphantes alticeps_COI_AY078 TATTATTAAT ATGCGGGGGT ATGGAGTCTC TATAGAAAAA GTTCCTCTTT TTGTGTGATC TGTATTAATT ACGGCTGTTT TGTTACTTTT ATCTCTTCCT
Frontinella zhui_COI_DQ396874 TATTTTAAAT ATACGAATAT ATGGAATTTC TATAGAAAAG GTGTCTTTAT TTGTATGATC TGTTTTGATT ACGGCAGTAT TGTTACTGTT ATCTTTACCT
Grammonota texana_COI_DQ029222 TATTATTAAT ATACGAGGAT ATGGTATAAC TATAGAAAAA GTTCCTTTAT TTGTTTGATC TGTATTAATC ACAGCTGTTT TATTATTATT ATCTTTACCA
Lepthyphantes minutus_COI_AY07 AATCATTAAT ATACGAGGAC ATGAGATATC TTTAGAAAAG ATTCCTTTAT TTGTTTGATC AGTTTTAATT ACGGCTGTTT TGTTATTATT ATCTTTACCT
Labulla thoracica_COI_AY078694 AATTTTGAAT ATACGAATAG AGAGTATTAG TATAGAAAAG ATATCTTTAT TTGTATGGTC TGTTTTAATT ACGGCAGTTT TATTATTATT ATCTTTACCT
Linyphia triangularis_COI_AY07 TATTCTAAAT ATACGGTCTT ATGGTGTTAG AATGGAGAAG GTTTCTTTAT TCGTTTGATC TGTTTTAATT ACTGCTGTAC TGTTATTGTT ATCTTTACCT
Microlinyphia dana_COI_AY07869 TATTTTAAAT ATGCGGGCTT ATGGAATTAG AATAGAAAAG GTGTCTCTAT TTGTTTGATC AGTTTTAATT ACTGCTGTTC TTTTGCTATT ATCTTTACCT
Microlinyphia mandibulata_COI_ TATTATAAAT ATACGAGTGT ATAGAATTAG AATAGAGAAG GTTTCTTTAT TTGTGTGATC AGTTTTGATC ACAGCTGTTC TTTTATTATT GTCTCTTCCT
Arion_ater_517_COI_AY987870 TATTTTTAAT ATGCGCCCCC AAGCCCTAAC ATTGGAGCGT ATAAGCTTAT TTGTTTGATC CATTTTGATT ACGGTTTTCT TACTACTACT GTCTTTACCT
Arion_hortensis_AH26_COI_AY423 TATTTTTAAC ATACGGCCAA AAGTATTAAC TTTTGAACGG TTAAGTTTAT TTGTTTGGTC TATTTTAATT ACCGTATTTC TTTTACTCCT CTCATTGCCG
Neriene cavaleriei_COI_DQ39686 AATTTTTAAT ATGCGAGCTT ATGGGGTTAC TATAGAAAAA GTTCCTTTAT TTGTGTGGTC GGTGTTAATT ACTGCTGTAC TTTTGTTGTT ATCGTTACCT
Neriene clathrata_COI_DQ396869 TATTTTAAAT ATACGTGTTT ATGGAATATC TATGGAGAAG GTTTCTTTAT TTGTTTGATC AGTTTTAATT ACTGCTGTTT TATTATTATT ATCTCTTCCA
Orsonwelles ambersonorum_COI_A TATTTTAAAT ATACGAGTTG AAGGGGTTAG TATGGAAAAG GTTTCTTTGT TTGTTTGATC TGTTTTAATT ACAGCTGTTC TTTTGTTGTT ATCATTACCA
Pityohyphantes costatus_COI_AY AATTTTAAAT ATACGAGCAT ATATAATTAG TATGGAAAAG GTGTCCTTAT TTGTTTGATC TATTTTAATT ACTGCTGTCC TTTTATTATT GTCTTTACCT
Pardosa_palustris_COI_DQ180943 TATCATTAAT ATACGAATAT TAGGAATATC TATAGAAAAG GTTCCTCTTT TTGTTTGATC AGTTTTAATT ACAGCAGTAT TATTGTTACT TTCTTTGCCT
Drassodes lapidosus_COI_AY5607 TATTGTAAAT ATACGAATAT TAGGAATAAG AATAGAGAAG GTTTCATTAT TTGTATGATC TGTATTAATT ACTGCTGTAT TATTATTATT ATCTCTACCA
Enoplognatha ovata_COI_DQ12744 TATTTTAAAT ATACGTTCTT TTGGAATAAG AATAGAAAAG GCTTCTCCTT TTGTCTGATC TGTTCTTATT ACTGCTGGTT TATTGTTACT TTCTTTACCT
Frontinella_communis_COI_DQ127 TATTTTAAAT ATACGAGGAT ATGGTGTTTC TATGGATAAA ATTTCTCTTT TTGTATGATC TGTATTAATT ACAGCTGTTC TTTTACTTTT ATCCTTACCT
Pasilobus hupingensis_COI_DQ51 TATTATTAAT ATACGATATT ATGGAATATC TATAGAAAAA GTACCTTTAT TTGTTTGATC TGTTTTAATT ACAGCTGTAT TATTATTATT ATCATTACCT
Bembidion nigripes_COI_DQ05978 AATTATTAAT ATACGATCAG TAGGAATAAC ATTTGATCGC ATGCCTTTAT TTGTATGATC AGTAGGAATT ACTGCTTTAT TATTATTACT ATCTTTACCA
P_melanarius_COI_DQ063219 AATTATTAAT ATACGATCAA TTGGAATAAC TTTTGATCGA ATACCATTAT TTGTTTGATC AGTAGGTATT ACTGCTTTAT TATTATTACT TTCATTACCT
Loricera_pilicornis_B_COI AATTATTAAT ATACGATCTA TTGGAATGAC ATTTGATCGA ATACCACTAT TTGTTTGATC AGTGGGAATT ACTGCTCTAC TTCTTTTACT TTCGTTACCA
Loricera_pilicornis_A_COI AATTATTAAT ATACGATCTA TTGGAATGAC ATTTGATCGA ATACCACTAT TTGTTTGATC AGTGGGAATT ACTGCTCTAC TTCTTTTACT TTCGTTACCA
Notiophilus_biguttatus_A_COI TATTATTAAT ATACGATCAG TTGGGATATC ATTTGATCGA ATACCCTTAT TTGTATGATC AGTTGGAATT ACAGCTCTTT TATTACTTTT ATCCTTACCT
Notiophilus_biguttatus_B_COI TATTATTAAT ATACGATCTG TAGGAATAAC ATTTGATCGA ATACCATTAT TTGTTTGATC AGTTGGAATT ACTGCTTTAT TATTATTATT ATCTTTGCCT
Poecilus cupreus_COI_AY574578 AATTATTAAT ATACGATCTA TTGGAATAAC TTTTGATCGA ATACCTTTAT TTGTATGATC TGTTGGAATT ACTGCTTTAC TTTTACTTCT TTCATTACCA
Coccinella_undecimpunctata_COI AATTATAAAT ATACGCCCCT TTGGAATAAA TTTAGATAAA ACTCCTTTAT TTGTTTGATC AGTACTTATT ACTGCTATTT TATTACTGCT TTCATTACCA
Staphylinus_olens_COI CGTAATCAAC ATACGATCAA CAGGAATAAC ATTTGATCGA ATACCGCTAT TCGTTTGATC CGTAGTTATT ACCGCTCTAT TGCTTCTCCT ATCTTTGCCA
Sitobion avenae_COI_AY383540 AATTCTAAAC ATAATACCAA ATAATATAAA ATTAAATCAA ATTCCTCTTT TCCCTTGATC AATTTTAATT ACAGCTATTT TATTAATTTT ATCTTTACCA
Aporrectodea_caliginosa_COI_DQ TGTTATCAAT ATACGATGAA GGGGTCTACG ACTCGAACGA ATTCCCCTAT TTGTATGAGC TGTAGTTATT ACAGTTGTAT TACTTCTTTT ATCCTTACCA
Lumbricus_terrestris_COI_DQ092 CGTAATCAAC ATACGTTGAA GCGGGTTACG GCTAGAACGA ATCCCTCTGT TTGTCTGAGC TGTACTAATT ACGGTAGTTC TCCTCCTCCT ATCCCTTCCT
Bembidion_nigripes_COI_DQ05978 AATTATTAAT ATACGATCAG TAGGAATAAC ATTTGATCGC ATGCCTTTAT TTGTATGATC AGTAGGAATT ACTGCTTTAT TATTATTACT ATCTTTACCA
Lepidocyrtus_cyaneus_COI_AY383 TATTATTAAT ATACGAACCC CTGGGTTGTC TTGGGACCAA ACTCCGTTGT TTGTGTGGTC TGTGTTCCTT ACAGCAATTT TATTACTACT ATCCCTTCCA
Entomobrya_multifasciata_COI_A AATTATTAAT ATACGAACCC CTGGAATATC TTGAGATCAA ACCCCTCTTT TTGTTTGATC TGTTTTTTTA ACCGCAATTC TTCTTCTTCT TTCCCTTCCT
Isotoma_anglicana_COI_AY383534 AATTATTAAT ATACGAGCCG TTGGAATATC ATGAGATCGA ACCCCTTTAT TTGTGTGGTC AGTATTTTTA ACAGCAATTT TACTTTTACT ATCACTTCCT


610 620 630 640 650 660 670 680 690 700
Habronattus oregonensis_COI (f GTTTTGGCAG GGGCTATTAC TATATTGTTA ACAGATCGTA ATTTTAATAC ATCATTTTTT GACCCTGCTG GAGGAGGGGA TCCTATTTTG TTTCAACATT
Nephila clavata_COI (from AY45 GTATTGGCAG GTGCTATTAC AATATTATTG ACTGATCGAA ATTTTAATAC ATCATTTTTT GATCCTTCTG GAGGGGGGGA TCCAATTTTA TTTCAACATT
E_dentipalpis_COI_AY383538 GTGCTTGCAG GAGCTATCAC CATGCTTTTA ACTGATCGAA ATTTTAATAC TTCTTTTTTT GATCCTTCTG GAGGGGGTGA TCCTGTGTTA TTTCAACATT
Erigone atra_COI_AY383537 GTGCTTGCAG GAGCTATCAC TATGCTTTTA ACTGATCGAA ATTTTAATAC TTCTTTTTTT GACCCTTCTG GGGGGGGTGA TCCTGTGTTA TTTCAACATT
T_tenuis_COI_AY383539 GTTTTAGCAG GTGCTATTAC AATACTTTTA ACTGATCGAA ATTTTAATAC TTCATTTTTT GACCCTGCAG GAGGGGGAGA TCCAGTATTA TTCCAACATT
Bathyphantes_gracilis_COI GTTTTAGCAG GAGCTATTAC TATATTATTA ACTGATCGAA ATTTTAATAC TTCTTTTTTT GATCCAGCAG GAGGAGGG-- ---------- ----------
B_pallidus_COI_AY944732 GTTTTAGCAG GRGCTATCAC TATATTATTA ACTGATCGAA ATTTTAATAC ---------- ---------- ---------- ---------- ----------
Tenuiphantes_tenuis_2_COI GTTTTAGCAG GTGCTATTAC AATACTTTTA ACTGATCGAA ATTTTAATAC TTCATTT--- ---------- ---------- ---------- ----------
Tengella radiata_COI_DQ628622 GTATTAGCAG GTGCTATTAC TATATTATTA ACTGATCGGA ATTTTAATAC TTCATTTTTT GATCCTGCAG GGGGAGGAGA TCCTGTATTG TTTCAACATT
Bolyphantes alticeps_COI_AY078 GTTTTAGCAG GAGCAATTAC AATATTATTA ACAGATCGCA ATTTTAATAC TTCTTTTTTT GACCCAGCTG GAGGAGGTGA TCCAGTTTTG TTTCAACATT
Frontinella zhui_COI_DQ396874 GTTCTAGCTG GGGCTATTAC TATATTATTA ACAGATCGAA ATTTTAATAC ATCTTTTTTT GATCCTTCTG GTGGTGGGGA TCCTATTTTA TTTCAACATT
Grammonota texana_COI_DQ029222 GTATTGGCTG GTGCTATTAC TATACTTTTA ACTGATCGAA ATTTTAATAC TTCTTTTTTT GATCCATCGG GGGGAGGGGA TCCTGTTCTA TTCCAACATT
Lepthyphantes minutus_COI_AY07 GTTTTAGCGG GAGCAATTAC AATATTGCTT ACTGATCGAA ATTTTAATAC ATCTTTTTTT GACCCGGCAG GGGGAGGGGA TCCTGTTTTA TTTCAACATT
Labulla thoracica_COI_AY078694 GTTTTAGCAG GGGCTATTAC TATGTTGTTA ACAGATCGAA ATTTTAATAC CTCATTTTTT GATCCAGCAG GAGGGGGGGA TCCAATTTTA TTTCAACATT
Linyphia triangularis_COI_AY07 GTTTTAGCAG GGGCTATTAC TATGTTGTTA ACTGATCGAA ATTTTAATAC ATCTTTTTTT GACCCTGCGG GGGGAGGTGA TCCTATTTTA TTTCAACATT
Microlinyphia dana_COI_AY07869 GTTTTAGCTG GCGCTATTAC TATATTGTTA ACTGATCGAA ATTTTAATAC ATCTTTTTTT GACCCGGCGG GGGGTGGTGA TCCTATTTTA TTTCAACATT
Microlinyphia mandibulata_COI_ GTGTTAGCAG GGGCTATTAC TATGTTATNA ACTGATCGAA ATTT------ ---------- ---------- ---------- ---------- ----------
Arion_ater_517_COI_AY987870 GTATTGGCTG GAGCTATTAC CATATTATTA ACTGACCGTA ATTTTAATAC TAGATTTTTT GATC------ ---------- ---------- ---------- ----------
Arion_hortensis_AH26_COI_AY423 GTCTTAGCTG GAGCTATTAC GATATTGCTA ACCGATCGAA ATTTTAATAC AAGTTTTTTT GATCCTGCT- ---------- ---------- ---------- ----------
Neriene cavaleriei_COI_DQ39686 GTTTTGGCGG GGGCTATTAC AATACTTTTA ACGGATCGTA ATTTTAATAC TTCTTTTTTT GATCCAGCTG GAGGGGGGGA TCCTATTTTA TTTCAACATT
Neriene clathrata_COI_DQ396869 GTTTTGGCAG GGGCAATTAC TATATTATTA ACAGATCGAA ATTTTAATAC TTCTTTTTTT GATCCTGCAG GAGGAGGAGA TCCTATTTTA TTTCAACATT
Orsonwelles ambersonorum_COI_A GTTTTAGCAG GGGCAATTAC TATATTATTA ACTGATCGAA ATTTAAATAC TTCTTTTTTT GATCCTGCGG GTGGGGGAGA TCCTATTTTA TTTCAGCATT
Pityohyphantes costatus_COI_AY GTTTTAGCTG GGGCTATTAC TATACTATTA ACTGATCGAA ATTTTAATAC ATCATTTTTT GATCCTGCTG GAGGAGGGGA TCCTATTTTA TTTCAACATT
Pardosa_palustris_COI_DQ180943 GTTTTAGCAG GTGCTATTAC TATATTATTA ACGGATCGAA ATTTTAATAC CTCTTTTTTT GACCCTGCTG GTGGGGGGGA TCCAATTTTA TTTCAACATT
Drassodes lapidosus_COI_AY5607 GTTTTAGCTG GAGCAATTAC TATATTATTA ACTGATCGTA ATTTTAATAC TTCTTTTTTT GATCCAGCTG GTGGTGGTGA TCCAATTTTA TTTCAACATT
Enoplognatha ovata_COI_DQ12744 GTATTAGCAG GAGCTATTAC TATATTATTG ACGGATCGAA ATTTTAATAC TTCA------ ---------- ---------- ---------- ----------
Frontinella_communis_COI_DQ127 GTTTTAGCAG GTGCCATTAC TATATTGTTG ACTGATCGAA ATTTTAATAC TTCTTTTTTT GACCCTGCGG GAGGAGGGGA TCCTATTTTA ----------
Pasilobus hupingensis_COI_DQ51 GTTTTAGCAG GTGCTATTAC TATATTATTA ACTGACCGAA ATTTTAATAC TTCTTTTTTT GACCCTTCAG GAGGTGGAGA TCCTATTTTA TTTCAACATT
Bembidion nigripes_COI_DQ05978 GTATTAGCAG GAGCTATTAC TATACTATTA ACAGATCGAA ATTTAAATAC TTCCTTTTTT GACCCAGCTG GAGGAGGAGA CCCAATTTTA TACCAACATT
P_melanarius_COI_DQ063219 GTATTAGCAG GAGCAATTAC TATATTATTA ACAGATCGAA ATTTAAATAC TTCATTTTTT GACCCAGCTG GAGGAGGAGA CCCGATTTTA TATCAACATT
Loricera_pilicornis_B_COI GTATTAGCTG GAGCAATTAC TATATTATTA ACTGATCGAA ATTTAAATAC ATCC------ ---------- ---------- ---------- ----------
Loricera_pilicornis_A_COI GTATTAGCTG GAGCAATTAC TATATTATTA ACTGATCGAA ATTTAAATAC ATCC------ ---------- ---------- ---------- ----------
Notiophilus_biguttatus_A_COI GTTTTAGCTG GAGCAATTAC TATATTATTA ACTGATCGAA ACTTAAATAC TTCTTTC--- ---------- ---------- ---------- ----------
Notiophilus_biguttatus_B_COI GTATTAGCAG GAGCTATTAC TAT------- ---------- ---------- ---------- ---------- ---------- ----------
Poecilus cupreus_COI_AY574578 GTATTAGCAG GAGCAATTAC TATACTATTA ACAGATCGAA ATTTAAATAC CTCTTTTTTT GATCCAGCAG GAGGAGGAGA TCCTATTTTA TATCAACATT
Coccinella_undecimpunctata_COI GTTTTAGCTG GGGCTATTAC AATACTGTTA ACTGACCGTA ATATTAATAC ATCTTTTTTT GATCCTACAG GAGGAGGTGA TCCAATTT-- ----------
Staphylinus_olens_COI GTCTTAGCTG GAGCAATTAC TATACTTTTA ACTGACCGAA ATCTTAATAC CTCATTTTTT G---------- ---------- ----------
Sitobion avenae_COI_AY383540 GTTTTAGCTG GTGCTATTAC AATATTATTA ACTGATCGAA ATCTAAATAC ATCATTTTTT GATCCAGCAG GAGGAGGAGA TCCAATTTTA TATCAACATT
Aporrectodea_caliginosa_COI_DQ GTCCTAGCAG GAGCTATTAC AATACTTTTA ACAGACCGAA ATCTAAATAC ATCATTTTTT GACCCGGCCG GAGGTGGGGA CCCTATTCTC TACCAACATC
Lumbricus_terrestris_COI_DQ092 GTACTTGCCG GAGCAATCAC AATGCTCCTA ACAGATCGAA ATCTTAATAC TTCATTTTTC GACCCCGCTG GTGGGGGGGA TCCAATTTTA TATCAACACC
Bembidion_nigripes_COI_DQ05978 GTATTAGCAG GAGCTATTAC TATACTATTA ACAGATCGAA ATTTAAATAC TTCCTTTTTT GACCCAGCTG GAGGAGGAGA CCCAATTTTA TACCAACATT
Lepidocyrtus_cyaneus_COI_AY383 GTGCTAGCAG GCGCCATTAC AATACTTTTA ACAGATCGTA ATCTAAACAC TTCTTTTTTC GACCCTGCCG GCGGGGGAGA CCCTATTTTA TACCAA----
Entomobrya_multifasciata_COI_A GTTTTAGCAG GAGCTATTAC CATACTTTTA ACAGATCGAA ATCTAAATAC TTCATTTTTT GACCCTGCTG GAGGAGGGGA TCCTATTTTA TATCAACATT
Isotoma_anglicana_COI_AY383534 GTGCTAGCGG GGGCAATTAC TATATTGTTG ACCGATCGAA ACTTAAATAC ATCATTTTTT GACCCGGCCG GTGGTGGGGA TCCTATCTTA TACCAACACT


710 720 730 740 750 760 770 780 790 800
Habronattus oregonensis_COI (f TATTTTGATT TTTTGGTCAT CCAGAAGTGT ATATTCTAAT TTT--GCCTG GATTTGGAAT TGTTTCCCAT GTAATTAGTG CTTCTGTTGG AAAGCGGGAA
Nephila clavata_COI (from AY45 TGTTTTGATT TTTTGGTCAT CCTGAAGTTT ATATTTTGAT TCT--TCCTG GATTTGGAAT TGTTTCTCAT ATTATTAGTA GTTCAGTAGG TAAGCGAGAA
E_dentipalpis_COI_AY383538 TATTTTGATT TTTTGGACAC CCAGAAGTTT ATATTTTAAT TTT--ACC--
Erigone atra_COI_AY383537 TATTTTGATT TTTTGGGCAC CCAGAAGTTT ATATTTTAAT TTT--ACCGG GA--------
T_tenuis_COI_AY383539 TATTTTGATT TTTTGGTCAT CCTGAAGTTT ATATTTTAAT TTT--ACCGG GA--------
Bathyphantes_gracilis_COI
B_pallidus_COI_AY944732
Tenuiphantes_tenuis_2_COI
Tengella radiata_COI_DQ628622 TATTTTGGTT TTTTGGACAT CCTGAAGTTT ATATTTTAAT TTT--ACCGG GA--------
Bolyphantes alticeps_COI_AY078 TATTT-----
Frontinella zhui_COI_DQ396874 TATTTTGATT TTTTGGGCAT CCTGAAGTTT ATATTTTAAT TTTT-ACCGG GGA-------
Grammonota texana_COI_DQ029222 TATTTTGATT TTTTGGTCAT CCTGAGGTTT ATATTTTAAT TTT--ACCTG GATTTGGGAT TGTTTCTCAT ATCATTAGTG CTTCTGTAGG TAAGCGTGAG
Lepthyphantes minutus_COI_AY07 TATT------
Labulla thoracica_COI_AY078694 TATT------
Linyphia triangularis_COI_AY07 TATTT-----
Microlinyphia dana_COI_AY07869 TATTT-----
Microlinyphia mandibulata_COI_
Arion_ater_517_COI_AY987870
Arion_hortensis_AH26_COI_AY423
Neriene cavaleriei_COI_DQ39686 TGTTCTGATT TTTTGGGCAT CCAGAAGTTT ATATTTTAAT TTT--ACCGG GA--------
Neriene clathrata_COI_DQ396869 TATTTTGATT TTTTGGGCAT CCTGAAGTTT ATATTTTATT TTTTTACCGG GGA-------
Orsonwelles ambersonorum_COI_A TGTTTTGATT TTTTGGGCAT CGT-------
Pityohyphantes costatus_COI_AY TATTTTGATT TTTTGGACAT CCT-------
Pardosa_palustris_COI_DQ180943 TATTTTGATT TTTTGGACAT CCAGAAGTTT ATATTTTAAT TTT--ACCGG G---------
Drassodes lapidosus_COI_AY5607 TATTTTGATT TTTTGGTCA-
Enoplognatha ovata_COI_DQ12744
Frontinella_communis_COI_DQ127
Pasilobus hupingensis_COI_DQ51 TATTTTGATT TTTTGGAAA-
Bembidion nigripes_COI_DQ05978 TATTTTGATT CTTTGGTCAT CCAGAAGTTT ATATTTTAAT TCT--ACCAG GATTTGGAAT AATTTCTCAC ATTATTAGCC AAGAAAGTGG AAAAAAGGAA
P_melanarius_COI_DQ063219 TATTTTGATT TTTTGGCCAT CCA-------
Loricera_pilicornis_B_COI
Loricera_pilicornis_A_COI
Notiophilus_biguttatus_A_COI
Notiophilus_biguttatus_B_COI
Poecilus cupreus_COI_AY574578 TATTTTGATT TTTTGGACAT CCAGAAGTTT ATATTTTAAT TTT--ACCTG GATTTGGTAT AATTTCTCAT ATTATTAGTC AAGAAAGAGG AAAAAAGGAA
Coccinella_undecimpunctata_COI
Staphylinus_olens_COI
Sitobion avenae_COI_AY383540 TATTTTGATT TTTTGGACAT CCTGAAGTTT ATATTTTAAT TTT--ACCGG
Aporrectodea_caliginosa_COI_DQ TATTT-----
Lumbricus_terrestris_COI_DQ092 TTTTC-----
Bembidion_nigripes_COI_DQ05978 TATTTTGATT CTTTGGTCAT CCAGAAGTTT ATATTTTAAT TCT--ACCAG GATTTGGAAT AATTTCTCAC ATTATTAGCC AAGAAAGTGG AAAAAAGGAA
Lepidocyrtus_cyaneus_COI_AY383
Entomobrya_multifasciata_COI_A TATTTTGATT TTTTGGTCAC CC--------
Isotoma_anglicana_COI_AY383534 TATTTTGATT TTTTGGGCAC CCC-------

Appendix I. Alignment of COI sequences (5' - 3') carried out using BioEdit software (Ibis Biosciences, Carlsbad, CA, USA).

Appendix II

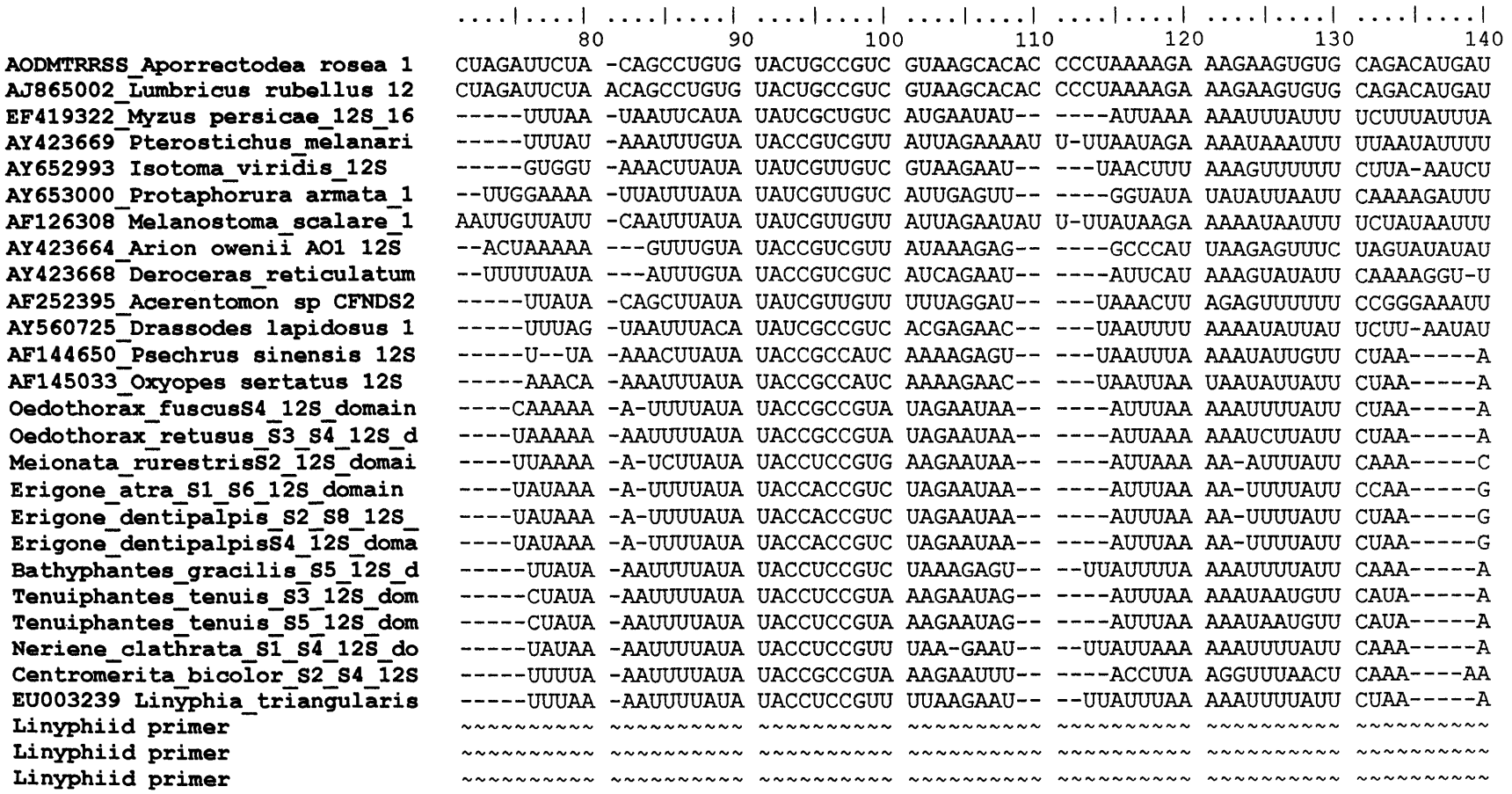
Species	Primer name	5'-3'	Length (bp)	GC%
Erigone atra, E. dentipalpis	Erigone_sp_C1-146F	GAA CAA TTT ATC CTC CTC TAG C	22	41
Erigone atra, E. dentipalpis	Erigone_sp_C1-185F	CTG GTA GTT CTG TTG ATT TTG CA	23	39
Erigone atra, E. dentipalpis	Erigone_sp_C1-389Rb	GTG ATA GCT CCT GCA AGC AC	20	55
Erigone atra	E_atra-C1-F1	GTA TAG ATG AGA TAG GTG TG	20	40
Erigone atra	E_atra-C1-R1	GAT AAT AAT AAC AGT ACA GCT	21	29
Erigone dentipalpis	E_denti-C1-F1	GAA TAG ATG AAA TAG GTG TA	20	30
Erigone dentipalpis	E_denti-C1-R1	GAT AAT AAT AAC AAT ACG GCT G	22	32
Bathyphantes gracilis	B_grac-C1-80F	GGA GAT GAC CAT TTA TAT AAT GTC	24	33
Bathyphantes gracilis	B_grac-C1-350R	AAT GCC CTT CTA AAG AAG CTA A	22	36
Bathyphantes gracilis	B_grac-C1-266R	TTA ATA ATG AAG GAG GGA GTA AC	23	35
Bathyphantes gracilis	B_grac-C1-163F	GGG GTT TGG TAA TTG GTT G	19	47
Bathyphantes gracilis	B_grac-C1-226F	GAA TAA TTT AAG ATT TTG GTT ACT C	25	24
Tenuiphantes tenuis	T_ten-C1-306F	GCT TCT TTG GAA GGT CAT GCA	21	48
Tenuiphantes tenuis	T_ten-C1-473R	CAC GGA TCA AAC AAA CAA TGG TAC	24	42
Tenuiphantes tenuis	T_ten-C1-153F	TTA GTT CCT TTG ATA CTT GGG GCA C	25	44
Pachygnatha degeeri	P_degeeri_C1-277F	GGC TAC TTC CCC CTT CGT TGT TC	23	57
Pachygnatha degeeri	P_degeeri_C1-399R	CTA CAG AGT TTC CAG AAT GCC CTT	24	46
Pachygnatha degeeri	P_degeeri_C1-233F	GGC TCC AGA TAT GGC TTT CCC TCG C	25	60
Pachygnatha degeeri	P_degeeri_C1-596R	TAG CAT TGT AAT AGC CCC CGC T	22	50

Species	Primer name	5'-3'	Length (bp)	GC%
Neriene clathrata	N_clathrata-C1-97F	GCA GGT TGA ACG GTT TAT CCA	21	48
Neriene clathrata	N_clathrata-C1-128F	CAT TAG AGG GAC ATT CTG GAA GA	23	43
Neriene clathrata	N_clathrata-C1-339R	CCC TGC CAA AAC AGG AAG AG	20	55
Pardosa amenensis, P. prativaga, P. palustris	Pardosa_C1-255F	CCT CGA ATA AAT AAT CTT TC	20	30
Pardosa amentata, P. prativaga, P. palustris	Pardosa_C1-593R	TAA AAC TGA TCA AAC AAA AAG	21	24
Oedothorax fuscus	O_fuscus_C1-138F	CTT CTC ATG CTT TTG TTA TG	20	35
Oedothorax fuscus	O_fuscus_C1-204F	GGT TAG TGC CTT TAA TGT TAG GT	23	39
Oedothorax fuscus	O_fuscus_C1-354R	GGA GGA TAA ATA GTC CAC CCG	21	52
Oedothorax apicatus	O_apicatus_C1-71F	ATT CGT ATT GAG TTA GGG C	19	42
Oedothorax apicatus	O_apicatus_C1-270F	GGT TGT TAC CCC CTT CTT TGG C	22	55
Oedothorax apicatus	O_apicatus_C1-309R	CTA GAA ACG AAT AAT AAA GCC	21	33
Oedothorax apicatus	O_apicatus_C1-571R	CGC TAA GAC AGG CAA CGA GAG TAG C	25	56
Oedothorax apicatus	O_apicatus_C1-204F	GGT TAG TTC CAT TAA TGT TAG GT	23	35
Oedothorax apicatus	O_apicatus_C1-373R	CCC TTC CAA AGA AGC AAG AGG G	22	55
Oedothorax retusus	O_retusus_C1-253F	GAA TAA TCT AAG ATT TTG GCT G	22	32
Oedothorax retusus	O_retusus_C1-270F	GGC TGT TAC CTC CTT CTT TGG T	22	50
Oedothorax retusus	O_retusus_C1-571R	CCC GCT AAA ACA GGC AAC GAA	21	52

Appendix II. A list of COI primers. Most are specific to one species, while a pair are designed to amplify all *Pardosa* species.

Alignment: D:\Sequences\Mitochondrial sequences\12S_16S\12S_domainIII_primer.fas

```
                                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                          10         20         30         40         50         60         70
AODMTRRSS_Aporrectodea rosea 1   -GGGCGGUGU CUUAU-CAAC CCAGGGGAAC CUGUCUCAUA ACUCGAUAAC CCACGAAUUC CUCACCCUCU
AJ865002_Lumbricus rubellus 12   -UGGCGGUGU CUUAU-CAAC CCAGGGGAAC CUGUCUCAUA ACUCGAUAAC CCACGAAUUC CUCACCCUCU
EF419322_Myzus persicae_12S_16   -UGGCGGUAU UUUAGUCUUA UUAGAGGAAC CUGUUUUUUA A-UUGAUAAU CCACGAUUUA UUUUACUUAU
AY423669 Pterostichus_melanari   -UGGCGGUAU UUUAGUCUAU UUAGAGGAAC CUGUUCUGUA A-UUGAUAAU CCACGAUUAA UUUUACUUAU
AY652993 Isotoma_viridis_12S     -UGGCGGUAU UUUAUUCUUU UCAGAGGAAC CUGUUCUAUA A-AAGAUACC ACACUA-UAA UUUUACCU--
AY653000_Protaphorura armata_1   -UGGCGGUAA AUACAUAUU- --AGAGGAAC CUGUUCUAUA A-UUGAUAAC CCACAGAA-A CUUAACCUUG
AF126308 Melanostoma_scalare_1   -UGGCGGUAU UUUAGUCUAU UCAGAGGAAC UUGUUUUUUA A-UCGAUAAU CCACGAUGUA CCUUACUUAA
AY423664_Arion owenii AO1 12S    -UGGCGGCAA AUGUAAACUA -CAGGGGAAC UUACUACAUA A-AUGAUAAU CACCAAGACA AACUACCUUA
AY423668 Deroceras_reticulatum   -UGGCGGCUG AGUUUAACUU -CAGGGGAAC UUACCAAAUA A-UAGAUAAC CACCAAGA-A GCUUCUCUUA
AF252395_Acerentomon sp CFNDS2   -UGGCGGUAU UUUAU-CUUA UCAGAGGAAC CUGUUUUUUA A-UGGACAAC ACACAAAUUA CUCUACUU--
AY560725_Drassodes lapidosus 1   -CGGCGGCA- AUAAACCUUA UUAGAGGAAC UUGUUAAAUA A-UCGACAAU ACACGAUGAA UUUUACUAAU
AF144650_Psechrus sinensis 12S   -UGGCGGCAU AACAU-CUAA UUAGAGGAAU UUGUCUAUUA A-UUGAUAAU CCACAAUAAA UUUUACUUUU
AF145033_Oxyopes sertatus 12S    -CGGCGGCGU UUUAU-UUUA CUAGAGGAAC UUGUUUAUUA A-UCGACAAC CCCCGAUAAA UUUUACUUUA
Oedothorax_fuscusS4_12S_domain   AUGGCGGCAU UUAUU--AAA UUAGAGGAAC CUGUCUAUUA A-UCGAUAAC CCACGAUUUA UCUUACUUUA
Oedothorax_retusus_S3_S4_12S_d   AUGGCGGCAU UUAUU--AAA UUAGAGGAAC CUGUUUAUUA A-UCGAUGGC CCACGAUUUA UCUCACUUUA
Meionata_rurestrisS2_12S_domai   CUGGCGGCAU UUAUC--UAA UUAGAGGAAU CUGUUUAUUA A-UCGAUAAC CCGCGAUAAA UUUCACUUUU
Erigone_atra_S1_S6_12S_domain    AUGGCGGCAU UUAUC--UAA UUAGAGGAAC CUGUUUAUAA A-UCGAUAAC CCACGUUAAA UCUUACUUUA
Erigone_dentipalpis_S2_S8_12S_   AUGGCGGCAU UUAUC--UAA UUAGAGGAAC CUGUUUAUAA A-UCGAUAAC CCACGUUAAA UCUUACUUUA
Erigone_dentipalpisS4_12S_doma   AUGGCGGCAU UUAUC--UAA UUAGAGGAAC CUGUUUAUAA A-UCGAUAAC CCACGUUAAA UCUUACUUUA
Bathyphantes_gracilis_S5_12S_d   AUGGCGGCAU UUUAU-CUCA UUAGAGGAAU CUGUUAAUUA A-UCGAUAAC CCACGAUAAA UUUUACUUUU
Tenuiphantes_tenuis_S3_12S_dom   CUGGCGGCAU UUUUU-CUAG UUAGAGGAAU CUGUUUAUUA A-UCGACAAC CCACGAUAAA UCUUACUUUU
Tenuiphantes_tenuis_S5_12S_dom   CUGGCGGCAU UUUUU-CUAG UUAGAGGAAU CUGUUUAUUA A-UCGACAAC CCACGAUAAA UCUUACUUUU
Neriene_clathrata_S1_S4_12S_do   AUGGCGGCAU UUUAU-CUAA UUAGAGGAAU CUGUUUAUUA A-UCGACAAU CCACGAUAAA UUUUACUUUU
Centromerita_bicolor_S2_S4_12S   CUGGCGGCAU UUUAU-CUUA UUAGAGGAAC CUGUUUAAUA A-UCGACAAC CCACGAUAAA UCUCACUUUA
EU003239 Linyphia_triangularis   AUGGCGGCAU UUUAU-CUAA UUAGAGGAAU CUGUUUUUUA A-UCGAUAUC CCCCGAUAAA UUUUACUUAU
Linyphiid primer                 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
Linyphiid primer                 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
Linyphiid primer                 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
```


....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
80 90 100 110 120 130 140
AODMTRRSS_Aporrectodea rosea 1 CUAGAUUCUA -CAGCCUGUG UACUGCCGUC GUAAGCACAC CCCUAAAAGA AAGAAGUGUG CAGACAUGAU
AJ865002_Lumbricus rubellus 12 CUAGAUUCUA ACAGCCUGUG UACUGCCGUC GUAAGCACAC CCCUAAAAGA AAGAAGUGUG CAGACAUGAU
EF419322_Myzus persicae_12S_16 -----UUUAA -UAAUUCAUA UAUCGCUGUC AUGAAUAU-- ----AUUAAA AAAUUUAUUU UCUUUAUUUA
AY423669 Pterostichus_melanari -----UUUAU -AAAUUUGUA UACCGUCGUU AUUAGAAAAU U-UUAAUAGA AAAUAAAUUU UUAAUAUUUU
AY652993 Isotoma_viridis_12S -----GUGGU -AAACUUAUA UAUCGUUGUC GUAAGAAU-- ---UAACUUU AAAGUUUUUU CUUA-AAUCU
AY653000_Protaphorura armata_1 --UUGGAAAA -UUAUUUAUA UAUCGUUGUC AUUGAGUU-- ----GGUAUA UAUAUUAAUU CAAAAGAUUU
AF126308 Melanostoma_scalare_1 AAUUGUUAUU -CAAUUUAUA UAUCGUUGUU AUUAGAAUAU U-UUAUAAGA AAAAUAAUUU UCUAUAAUUU
AY423664_Arion owenii AO1 12S --ACUAAAAA ---GUUUGUA UACCGUCGUU AUAAAGAG-- ----GCCCAU UAAGAGUUUC UAGUAUAUAU
AY423668 Deroceras_reticulatum --UUUUUAUA ---AUUUGUA UACCGUCGUC AUCAGAAU-- ----AUUCAU AAAGUAUAUU CAAAAGGU-U
AF252395_Acerentomon sp CFNDS2 -----UUAUA -CAGCUUAUA UAUCGUUGUU UUUAGGAU-- ---UAAACUU AGAGUUUUUU CCGGGAAAUU
AY560725_Drassodes lapidosus 1 -----UUUAG -UAAUUUACA UAUCGCCGUC ACGAGAAC-- ---UAAUUUU AAAAUAUUAU UCUU-AAUAU
AF144650_Psechrus sinensis 12S -----U--UA -AAACUUAUA UACCGCCAUC AAAAGAGU-- ---UAAUUUA AAAUAUUGUU CUAA-----A
AF145033_Oxyopes sertatus 12S -----AAACA -AAAUUUAUA UACCGCCAUC AAAAGAAC-- ---UAAUUAA UAAUAUUAUU CUAA-----A
Oedothorax_fuscusS4_12S_domain ----CAAAAA -A-UUUUAUA UACCGCCGUA UAGAAUAA-- ----AUUUAA AAAUUUUAUU CUAA-----A
Oedothorax_retusus_S3_S4_12S_d ----UAAAAA -AAUUUUAUA UACCGCCGUA UAGAAUAA-- ----AUUAAA AAAUCUUAUU CUAA-----A
Meionata_rurestrisS2_12S_domai ----UUAAAA -A-UCUUAUA UACCUCCGUG AAGAAUAA-- ----AUUAAA AA-AUUUAUU CAAA-----C
Erigone_atra_S1_S6_12S_domain ----UAUAAA -A-UUUUAUA UACCACCGUC UAGAAUAA-- ----AUUUAA AA-UUUUAUU CCAA-----G
Erigone_dentipalpis_S2_S8_12S_ ----UAUAAA -A-UUUUAUA UACCACCGUC UAGAAUAA-- ----AUUUAA AA-UUUUAUU CUAA-----G
Erigone_dentipalpisS4_12S_doma ----UAUAAA -A-UUUUAUA UACCACCGUC UAGAAUAA-- ----AUUUAA AA-UUUUAUU CUAA-----G
Bathyphantes_gracilis_S5_12S_d -----UUAUA -AAUUUUAUA UACCUCCGUC UAAAGAGU-- --UUAUUUUA AAAUUUUAUU CAAA-----A
Tenuiphantes_tenuis_S3_12S_dom -----CUAUA -AAUUUUAUA UACCUCCGUA AAGAAUAG-- ----AUUUAA AAAUAAUGUU CAUA-----A
Tenuiphantes_tenuis_S5_12S_dom -----CUAUA -AAUUUUAUA UACCUCCGUA AAGAAUAG-- ----AUUUAA AAAUAAUGUU CAUA-----A
Neriene_clathrata_S1_S4_12S_do -----UAUAA -AAUUUUAUA UACCUCCGUU UAA-GAAU-- --UUAUUAAA AAAUUUUAUU CAAA-----A
Centromerita_bicolor_S2_S4_12S -----UUUUA -AAUUUUAUA UACCGCCGUA AAGAAUUU-- ----ACCUUA AGGUUUAACU CAAA----AA
EU003239 Linyphia_triangularis -----UUUAA -AAUUUUAUA UACCUCCGUU UUAAGAAU-- --UUAUUUAA AAAUUUUAUU CUAA-----A
Linyphiid primer ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
Linyphiid primer ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
Linyphiid primer ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

```
                                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                          150        160        170        180        190        200        210
AODMTRRSS_Aporrectodea rosea 1    UAAACUCAUA -UACGUCAGG UCAAAGUGCA GCCCAUGGGA GGGA-GAUGA UGGGUUACAC CCUAAAC-AA
AJ865002_Lumbricus rubellus 12    UAAACUCAUA -UACGUCAGG UCAAAGUGCA GCCCAUGGGA GGGA-GAUGA UGGGUUACAC CCUAAAC-AA
EF419322_Myzus persicae_12S_16    UUUUUGUAAU UUAUGUUAAG UCAAGAUGUG GUUUAUAAAU AAGUAUUUAA UGGGUUACAU UAAAUUU--U
AY423669 Pterostichus_melanari    AUAUUAAAAA AUAUAUCAGA UCAAGGUGCA GUAGAUAUUU AAGA-AGAAA UGGGUUACAU UAAAAAU-AU
AY652993 Isotoma_viridis_12S      UAAUGAGUUU -UAUGUCAAA UCUAGGUGUA GU--GUUUAC AGGUUAGAAA UGGGUUACUU UUAUUUA--A
AY653000_Protaphorura armata_1    AAUAAUAGAA -AAGAUCAAA UCAAGGUGUA GUUUAAUAAU AAGGAAGAAA UGGGUUACAU AUAUUAA--A
AF126308 Melanostoma_scalare_1    UAAAAAAAAU UAAUAUCAAA UCAAGGUGUA GUUUAUUUUU AAGU-AUAAA UGAGUUACAA UAAAAUU--U
AY423664_Arion owenii AO1 12S     UAAACUAUAA -GAUAACAGA UCAAGGUGCA ACCUAUGCUA AGGUUGUCAG CGAGUUACAA UAAAUAU--U
AY423668 Deroceras_reticulatum    UA---UACAA -AAUGACAGA UCAUGGUGCA GUAAACAAAU AAGUUACUGG CGAGUUACAA UAACUAA--G
AF252395_Acerentomon sp CFNDS2    UAAUUUAUAA -AACAUCAAA UCAAGGUGUA GC--GUUUAU AAGA-AUUAA UGGGUUACAA UUACUUA--A
AY560725_Drassodes lapidosus 1    AAAAAUAAAA ---AGUCAGG UCAAGGUGUA GUUAACAAUU UAGA-UUAAA UGAGUUACCU UAAA-AA--U
AF144650_Psechrus sinensis 12S    UAAUUAUUAA AAGUUAUAGG UCAAGGUGUA GUUUAUAU-A AAGU-AUAGU UGAAUUACAU UAAAAUA-AA
AF145033_Oxyopes sertatus 12S     UAAAUUAUAA AA-UGUUAGG UCAAGGUGUA AUUAAUAUUA AAGA-AUAAA UGAGUUACAC UAAAAUUUAA
Oedothorax_fuscusS4_12S_domain    AAAUUUUUAA -AAAAAUAGG UAAUGGUGUA GACUAUACUA AAGA-UUAAA UGGGUUACCU ACGAAAA-UA
Oedothorax_retusus_S3_S4_12S_d    AAAUUUUUAA -AAAGAUAGG UAAUGGUGUA GACUAUACUA AAGA-UUAAA UGGGUUACUU AUAAAGA-UG
Meionata_rurestrisS2_12S_domai    UAAUAUUAAA -GAAAAUAGG UAAUGGUGUA GACUAUAUAA AAGA-UUAAA UGGAUUACUU AUGAAAA-UC
Erigone_atra_S1_S6_12S_domain     AAAUGUCUUA -AAAAAUAGG UAAUGGUGUA GACUAUAAUA AAGC-CUAAA UGGGUUACUU AUAAAAU-CU
Erigone_dentipalpis_S2_S8_12S_    AAAUAUCUUC -AAAAAUAGG UAAUGGUGUA GACUAUAAUA AAGC-CUAAG UGGGUUACUU AUAAAAU-CU
Erigone_dentipalpisS4_12S_doma    AAAUAUCUUC -AAAAAUAGG UAAUGGUGUA GACUAUAAUA AAGC-CUAAG UGGGUUACUU AUAAAAU-CU
Bathyphantes_gracilis_S5_12S_d    UAAUUUAUAA -AAAGAUAGG UAAAGGUGUA GAUAACAAAA AAGA-UUUUA UGGAUUACA- UUAAGAA-UA
Tenuiphantes_tenuis_S3_12S_dom    AAAUGUAUAA -AAAAAUAGG UAAAGGUGUA GACUACAGAA AAGU-UUAAA UGGAUUACAU UAAACAU--U
Tenuiphantes_tenuis_S5_12S_dom    AAAUGUAUAA -AAAAAUAGG UAAAGGUGUA GACUACAGAA AAGU-UUAAA UGGAUUACAU UAAACAU--U
Neriene_clathrata_S1_S4_12S_do    AACUUUCUAA -AAAAAUAGG UAAAGGUGUA GACUACUAAA AAGA-AUAUA UGGAUUACAC UUAAAAA-UU
Centromerita_bicolor_S2_S4_12S    AACUCUAUAA -AAAUAUAGG UAAAGGUGUA GACCACAAUA UAGA-UCAAA UGGGUUACAU UAAAAAA--A
EU003239 Linyphia_triangularis    AAAUUUUUAA -AAAAAUAGG UAAAGGUGUA GACCACAAUU AAGU-UAAAA UGGGUUACA- AUAAAUU-CU
Linyphiid primer                  ~~~~~~~~~~ ~~~~~AUAGG UAAYggUGUA GA..... ..... ..... .....
Linyphiid primer                  ~~~~~~~~~~ ~~~~~AUAGG UAAAGGUGUA GA..... ..... ..... .....
Linyphiid primer                  ~~~~~~~~~~ ~~~~~AUAGG UAAAGGUGUA GA..... ..... ..... .....
```

```
                                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       220        230        240        250        260        270        280
AODMTRRSS_Aporrectodea rosea 1   AGAUACGGAA UAUAGUACUA AAAGCUAUAU AA--AUUAUU ACUUGGUUGU AAC---GUUU CUUCAAAACU
AJ865002_Lumbricus rubellus 12   AGAUACGGAA UAUAGUACUA AAAGCUAUAU AA--AGGAGG ACUUGGUUGU AAC---GUUU CUUCAAAACU
EF419322_Myzus persicae_12S_16   AUUUAUGGAU UUUAAA-UGA AAAUUAAAAU GA--AAUUGG AUUUAAUAGU AAA--UUUAU UUAUUUAAUU
AY423669 Pterostichus_melanari   UUAUAUGGAU UAAAGUAAGA AUAAAUUUUA UAA-AGGUGG AUUUAAUAGU AAU--AAGAU UAAUUUAAAU
AY652993 Isotoma_viridis_12S     AUAAUU--UU UUUAGCCUUA GAGAUCUAAG AAA-AAUUGG AUUUGAAAGU AAU--CUU-U AAAUUUAUCU
AY653000_Protaphorura armata_1   AUAAU--AGA UUGGAGUUGA AAGAUUAAAU AA--AAAAGG AUUUA-AUGU AUA--UAUAG AAAAUUAUAU
AF126308 Melanostoma_scalare_1   AUUUUUGGAU UUAAAUAUGA AAAAUUUAAU GA--AAUUGG AUUUGAUAGU AAA--AUUAU AAAGAUAAAU
AY423664_Arion owenii AO1 12S    AUUUCCCACA UAAGGAUUAA UUAUCCUUAU AA--AGAUGG ACUUGUAAGU AUA--UUAUA UUAUAAUUAA
AY423668 Deroceras_reticulatum   AUUUCGAAAG CCAA-AUUAA UUUAAGUUG- -A--AGCUGG ACUUGAAAGU AAA--UUUAA UUUUAUUUAA
AF252395_Acerentomon sp CFNDS2   GUAAUAGACU UUUAGU-UUA AAUCUGUCAG AAC-AAU-GG AUUUGAAAGU AAU--UUU-G ----UUAGGU
AY560725_Drassodes lapidosus 1   AUUUAAGGAU UAGUUAAUAA UUAAUUAAUU AUUAAAAAGG AUUUAAAAAC AAU--UUUUU AAAACAUUUU
AF144650_Psechrus sinensis 12S   UUUAAGGAUU UUUUUUAUUA AAAUUUUAAA UGAAAAUAGG AUUUAAAAGU AAAAAUUUAU AAUAAUAAUA
AF145033_Oxyopes sertatus 12S    UUAAAGAACA UUAUUUUUUA AUAUUUUUAA --GAAAUAGG AUUUAGAAGU AAA--UUCUU AAUAAUAUUU
Oedothorax_fuscusS4_12S_domain   AUUCAAAGAU UAAAUUUUUA ACAAACCAAA AA--AUAAGG AUUUAAAAGU AAU--UAAAA AAUAAUAAUU
Oedothorax_retusus_S3_S4_12S_d   AUUUAAAGAU CUAACUUUAA AC--CCAACA GA--AUGAGG AUUUAAAAGU AAU--UAAAA AUCAAUAACU
Meionata_rurestrisS2_12S_domai   AUUUAAAUAA UAUAAUUGAA AUAAAUUAGA -A--AUUAGG AUUUAAAAGU AAU--UAAAA AAAAUUAUUU
Erigone_atra_S1_S6_12S_domain    AUUUAAAAAU UGAUUCUAAA AUUAAAAAAA GA--AUAAGG AUUUAAAAGU AAU--UUAGA AAUAAUAUUU
Erigone_dentipalpis_S2_S8_12S_   AUUUAAAAAU UGAUUAUAAA AUUAAAAAAA GA--AUAAGG AUUUAAAAGU AAU--UUAGA AAUAAUAUUU
Erigone_dentipalpisS4_12S_doma   AUUUAAAAAU UGAUUAUAAA AUUAAAAAAA GA--AUAAGG AUUUAAAAGU AAU--UUAGA AAUAAUAUUU
Bathyphantes_gracilis_S5_12S_d   ACUUUAGGAU A-UAAUCUUA AACCAUUAUU CA--AAAAGG AUUUAAAAGU AAAAUUAUUA AUAAUAUUAA
Tenuiphantes_tenuis_S3_12S_dom   AUUUAAGGAC AUAUAAUUAA ACUAAUACCU AA---UAAGG AUUUAAAAGU AAU--UUUAA AAUAGUAUUU
Tenuiphantes_tenuis_S5_12S_dom   AUUUAAGGAC AUAUAAUUAA ACUAAUACCU AA---UAAGG AUUUAAAAGU AAU--UUUAA AAUAGUAUUU
Neriene_clathrata_S1_S4_12S_do   AUUUAAGGAU AAUAAACUAA UAAAUUUUUU UA--AAAAGG AUUUAAAAGU AAUUAUAA-A AUAAUAUAUU
Centromerita_bicolor_S2_S4_12S   AUUUAAGAAU AAAAUGUAAA UUUAAUUAAC GA---UAAGG AUUUAAAAGU AAU--UAUAA AAUAAUAUUU
EU003239 Linyphia_triangularis   AUUUAAGGAU U-UGAUUAUA AUAUAUCUCU AA--AAGGGG AUUUAAAAGU AAAUUUAU-A AUAAUAUUUU
Linyphiid primer                 .......... .......... .......... .......... .......... .......... ..........
Linyphiid primer                 .......... .......... .......... .......... .......... .......... ..........
Linyphiid primer                 .......... .......... .......... .......... .......... .......... ..........
```

```
                                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                          290        300        310        320        330        340        350
AODMTRRSS_Aporrectodea rosea 1    AAAGUGAAUA UGAAUCUAAG ---------- ---------- ---------- ---------- ----------
AJ865002_Lumbricus rubellus 12    AAAGUGAAUA UGAAUCUAAG ACAUGUACAC AUCGCCCGUC GCUCUU---- ---------- ----------
EF419322_Myzus persicae_12S_16    AAUAUGAAGA AAGAUCUAAA AUAUGUACAU AUUGCCCGUC AUUCUUAUUA AAAAUAAGAC AAGUCGUAAC
AY423669 Pterostichus_melanari    UUUAUGAUUU UAGCUCUAAA AUAUGU---- ---------- ---------- ---------- ----------
AY652993 Isotoma_viridis_12S      AAUUUGAAUU AAGCUCUAAA AU-------- ---------- ---------- ---------- ----------
AY653000_Protaphorura armata_1    AUAUGAAAU- AUUAAUAUUU AUU------- ---------- ---------- ---------- ----------
AF126308 Melanostoma_scalare_1    AAUUUGAUUU UAGCUCU--- ---------- ---------- ---------- ---------- ----------
AY423664_Arion owenii AO1 12S     UCAUAGAAUC AUUACACUAU AUUUGUGU-- ---------- ---------- ---------- ----------
AY423668 Deroceras_reticulatum    AUUUUGAAU- -UUAAAAAAG UUCAGUGU-- ---------- ---------- ---------- ----------
AF252395_Acerentomon sp CFNDS2    AA--UGAAUA AAGCUCUAAU G--------- ---------- ---------- ---------- ----------
AY560725_Drassodes lapidosus 1    AAAAUGAAAA AUAUUUUAAU GUGUACACAU CGCCCGUCGC U--------- ---------- ----------
AF144650_Psechrus sinensis 12S    AUUUUUGAAU UUGUUAG-AA AUGUACACAU AUCGCCCGUC AUCCUUUUUU UAAAAAAAGG U---------
AF145033_Oxyopes sertatus 12S     AAAUUUGAAU AAAAUAAUAA AUGUGCACAU AUCGCCCGUC ACCCUUAUCA UAAGAUAAGG U---------
Oedothorax_fuscusS4_12S_domain    UAUUAUAAAU AUUAGUAUAA AUGUGCACAU AUCGCCCGUC ACCCUCAUCC CAAGAUGAGG CAAGUCGUA-
Oedothorax_retusus_S3_S4_12S_d    UAUAAUAAAU AUUAAUAUAA AUGUGCACAU AUCGCCCGUC ACCCUCAUCC AAAGAUGAGG CAAGUCGUAA
Meionata_rurestrisS2_12S_domai    UCUAAUAAAU AUAUUAAUAA AUGUGCACAU AUCGCCCGUC ACCCUCAUCA CAAGAUGAGG UAAGUCGUAA
Erigone_atra_S1_S6_12S_domain     UUAAAUAAAU AUUAUUGUAA AUGUGCACAU AUCGCCCGUC ACCCUCAUCU CA-------- ----------
Erigone_dentipalpis_S2_S8_12S_    UUAAAUAAAU AUAAUUAUAA AUGUGCACAU AUCGCCCGUC ACCCUCAUCU CA-------- ----------
Erigone_dentipalpisS4_12S_doma    UUAAAUAAAU AUAAUUAUAA AUGUGUACAU AUCGCCCGUC ACCCUCAUCU CAAGAUUAGG UAAGUCGUAA
Bathyphantes_gracilis_S5_12S_d    AAUUCGAAU- AAGAUAAUAA AUGUGCACAU AUCGCCCGUC ACCCUUAUCU UAAGAUAAGG UAAGUCGUAA
Tenuiphantes_tenuis_S3_12S_dom    UAAAAUAAAU UAGAUUAAAA AUGUGUACAU AUCGCCCGUC ACCCUCAUCU CAAGAUGAGG UAAGUCGUAA
Tenuiphantes_tenuis_S5_12S_dom    UAAAAUAAAU UAGAUUAAAA AUGUGUACAU AUCGCCCGUC ACCCUCAUCU CAAGAUGAGG UAAGUCGUAA
Neriene_clathrata_S1_S4_12S_do    AUUUUGAAU- AAGAUAGCUA AUGUGCACAU AUCGCCCGUC ACCCUUAUCU AAAGAUAAGG UAAGUCGUAA
Centromerita_bicolor_S2_S4_12S    UAUAAUGGCU AAGAACAAAA AUGUGUACAU AUCGCCCGUC ACCCUCAUCU CAAGAUGAGG UAAGUCGUAA
EU003239 Linyphia_triangularis    AUUUUGAAU- AAGAUAAUAA AUGUGC---- ---------- ---------- ---------- ----------
Linyphiid primer                  .......... .......... .......... .......... .......... .......... ..........
Linyphiid primer                  .......... .......... .......... .......... .......... .......... ..........
Linyphiid primer                  .......... .......... .......... .......... .......... .......... ..........
```

Appendix IV

Species	Taxon
Araneus quadratus	Araneae: Araneidae
Bathyphantes gracilis	Araneae: Linyphiidae
Erigone atra	Araneae: Linyphiidae
Erigone dentipalpis	Araneae: Linyphiidae
Tenuiphantes tenuis	Araneae: Linyphiidae
Meioneta rurestris	Araneae: Linyphiidae
Oedothorax apicatus	Araneae: Linyphiidae
Oedothorax retusus	Araneae: Linyphiidae
Oedothorax fuscus	Araneae: Linyphiidae
Neriene clathrata	Araneae: Linyphiidae
Centromerita bicolor	Araneae: Linyphiidae
Pardosa amenensis	Araneae: Lycosidae
Pardosa prativaga	Araneae: Lycosidae
Pardosa palustris	Araneae: Lycosidae
Pisaura mirabilis	Araneae: Lycosidae
Trochosa ruricola	Araneae: Lycosidae
Pholcus phalangioides	Araneae: Pholcidae
Heliophanus sp. I	Araneae: Salticidae
Heliophanus sp. II	Araneae: Salticidae
Pachygnatha degeeri	Araneae: Tetragnathidae
Pachygnatha clercki	Araneae: Tetragnathidae
Tetragnatha monticola	Araneae: Tetragnathidae
Tetragnatha sp. I	Araneae: Tetragnathidae
Tetragnatha sp. II	Araneae: Tetragnathidae
Meta mengei	Araneae: Tetragnathidae
Enoplognatha ovata	Araneae: Theridiidae
Xysticus sp.	Araneae: Thomisidae
Amara aenea	Coleoptera: Carabidae
Bembidion lampros	Coleoptera: Carabidae
Bembidion obtusus	Coleoptera: Carabidae
Calathus fuscipes	Coleoptera: Carabidae
Clivinia fossor	Coleoptera: Carabidae
Demetrius atricapillus	Coleoptera: Carabidae
Harpalus rufipes	Coleoptera: Carabidae
Harpalus aenus	Coleoptera: Carabidae
Loricera pilicornis	Coleoptera: Carabidae
Nebria brevicollis	Coleoptera: Carabidae
Notiophilus biguttatus	Coleoptera: Carabidae
Poecilus cupreus	Coleoptera: Carabidae
Pterostichus madidus	Coleoptera: Carabidae
Pterostichus melanarius	Coleoptera: Carabidae
Coccinella septempunctata	Coleoptera: Coccinellidae

Species	Taxon
Arion owenii	Pulmonata: Arionidae
Limax flavus	Pulmonata: Limacidae
Limax maximus	Pulmonata: Limacidae
Deroceras reticulatum	Pulmonata: Limacoidea
Tandonia budapestensis	Pulmonata:
Arion sp. *(hortensis/sylvaticus)*	Pulmonata: Arionidae
Heterorhabditis megidis	Rhabditida: Heterorhabditidae
Steinernema feltiae	Rhabditida: Steinernematidae
Steinernema kraussei	Rhabditida: Steinernematidae
Phasmarhabditis hermaphrodita	Rhabditida: Rhabditidae
Lonchoptera furcata	Diptera: Lonchoperidae
Lonchoptera lutea	Diptera: Lonchoperidae
Scaptomyza pallida	Diptera: Parascaptomyza
Lotophila atra	Diptera: Sphaeroceridae
Pteremis fenestralis	Diptera: Sphaeroceridae
Phyligria stictica	Diptera: Ephydridae
Opomyza germinationis	Diptera: Opomyzidae
Opomyza florum	Diptera: Opomyzidae
Medetera sp.	Diptera: Doliopodidae
Drosophila andalusiaca	Diptera: Drosophilidae
Sitodiplosis mosellana	Diptera: Cecidiomidae
Scaeva pyrastri	Diptera: Syrphidae
Metasyrphus luniger	Diptera: Syrphidae
Melanostoma scalare	Diptera: Syrphidae
Lycoriella castanescens	Diptera: Sciaridae
Bradysia confinis	Diptera: Sciaridae
Mayetiola sp.	Diptera: Cecidiomidae
Megaselia sp.	Diptera: Phoridae
Campsicnemus cucivites	Diptera: Dolocopidae
Pteremis fenestralis	Diptera: Dolocopidae
Mesostigmatid mite sp. I	Mesostigmata
Mesostigmatid mite sp. II	Mesostigmata

Appendix IV. Species from which DNA was extracted and subsequently tested against for cross-reactivity of primers.

LINYPHIINE Species	FWD (score)	REV (score)	SIZE (bp)	Mean Tm	Tm DIFF	µM each primer	2x MPLEX	BSA	Q	primer mix	DNA
Tenuiphantes tenuis-M	306F (84)	450R (82)	145	59	2.6	5.0	5	0.1	na	1	1
Bathyphantes gracilis-L	80F (79)	350R (79)	271	54.2	3	7.5					
Neriene clathrata- S	128F (91)	339R (84)	212	58.9	1.7	2.5					

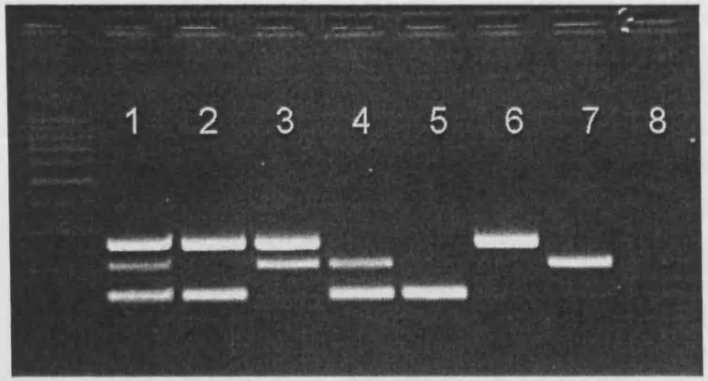


Fig.1. Results of PCR dropout testing for LINYPHIINE MPLEX. Annealing temperature = 60°C. Lane 1 contains all three species, lane 2 *B. gracilis* and *T. tenuis*, lane 3 *B. gracilis* and *N. clathrata*, lane 4 *N.clathrata* and *T. tenuis*, lane 5 *T. tenuis*, lane 6 *B. gracilis*, lane 7 *N.clathrata* and lane 8 = negative control.

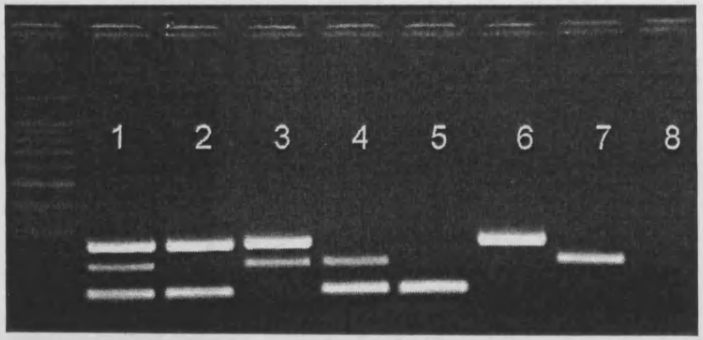


Fig. 2. Results of PCR dropout testing for LINYPHIINE MPLEX. Annealing temperature = 61°C. Lane 1 contains all three species, lane 2 *B. gracilis* and *T. tenuis*, lane 3 *B. gracilis* and *N. clathrata*, lane 4 *N.clathrata* and *T. tenuis*, lane 5 *T. tenuis*, lane 6 *B. gracilis*, lane 7 *N.clathrata* and lane 8 = negative control.

Species	FWD (score)	REV (score)	SIZE (bp)	Mean Tm	Tm DIFF	µM each primer	2x MPLEX	BSA	Q	primer mix	DNA
Tenuiphantes tenuis-M	306F (84)	450R (82)	145	59	2.6	2.5	5	0.1	na	1	1
Bathyphantes gracilis-L	80F (79)	350R (79)	271	54.2	3	5					
Neriene clathrata- S	128F (91)	339R (84)	212	58.9	1.7	2.5					

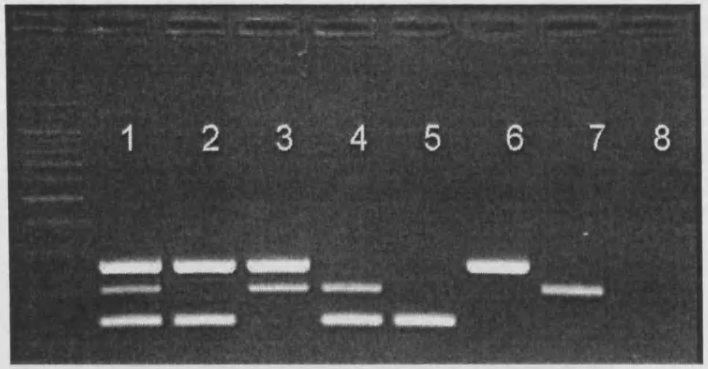


Fig.3 . Results of PCR dropout testing for LINYPHIINE MPLEX. Annealing temperature = 60°C. Lane 1 contains all three species, lane 2 *B. gracilis* and *T. tenuis*, lane 3 *B. gracilis* and *N. clathrata*, lane 4 *N.clathrata* and *T. tenuis*, lane 5 *T. tenuis*, lane 6 *B. gracilis*, lane 7 *N.clathrata* and lane 8 = negative control.

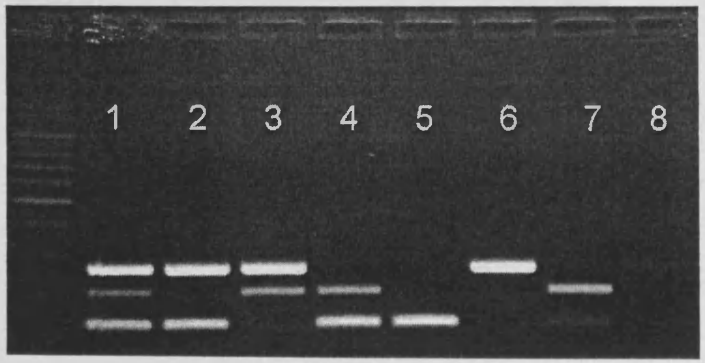


Fig. 4. Results of PCR dropout testing for LINYPHIINE MPLEX. Annealing temperature = 61°C. Lane 1 contains all three species, lane 2 *B. gracilis* and *T. tenuis*, lane 3 *B. gracilis* and *N. clathrata*, lane 4 *N.clathrata* and *T. tenuis*, lane 5 *T. tenuis*, lane 6 *B. gracilis*, lane 7 *N.clathrata* and lane 8 = negative control.

4x Linyphiids MPLEX

Species	FWD (score)	REV (score)	SIZE (bp)	Mean Tm	Tm DIFF	µM each primer	2x MPLEX	BSA	Q	primer mix	DNA
Bathyphantes gracilis-L	80F (79)	350R (79)	271	54.2	3	0.2	5	0.1	na	1	1
Neriene clathrata- L	97F (100)	339R (84)	243	58.9	1.7	0.2					
Oedothorax fuscus	204F (87)	354R (99)	149	56.5	3	0.8					
Tenuiphantes tenuis-S	320F (87)	439R (91)	120	59.2	1.6	0.2					

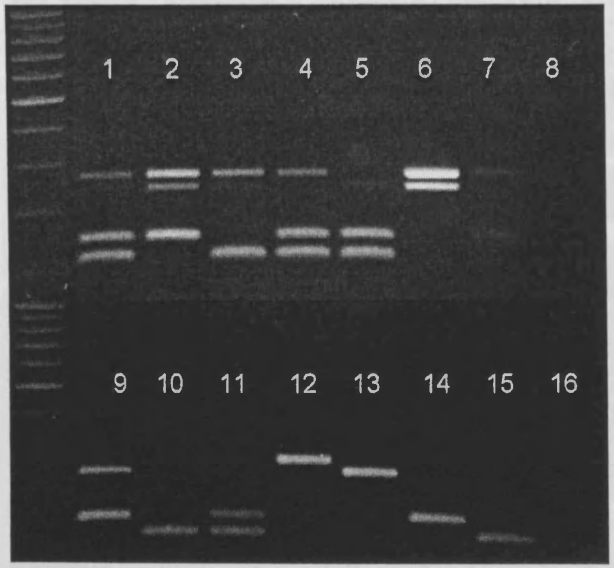


Fig. 5. Agarose gel image from PCR dropout testing of a multiplex with four primer pairs aiming to amplify four different species.

COM LINY MPLEX

Species	FWD (score)	REV (score)	SIZE (bp)	Mean Tm	Tm DIFF	µM each primer	2x MPLEX	BSA	Q	primer mix	DNA
Tenuiphantes tenuis-M	306F (84)	450R (82)	145	59	2.6	0.25	5	0.1	0.1	1	1
Bathyphantes gracilis-L	80F (79)	350R (79)	271	54.2	3	0.25					
Erigone sp. - *S*	185F (85)	389Rb(85)	205	56.6	2	0.25					

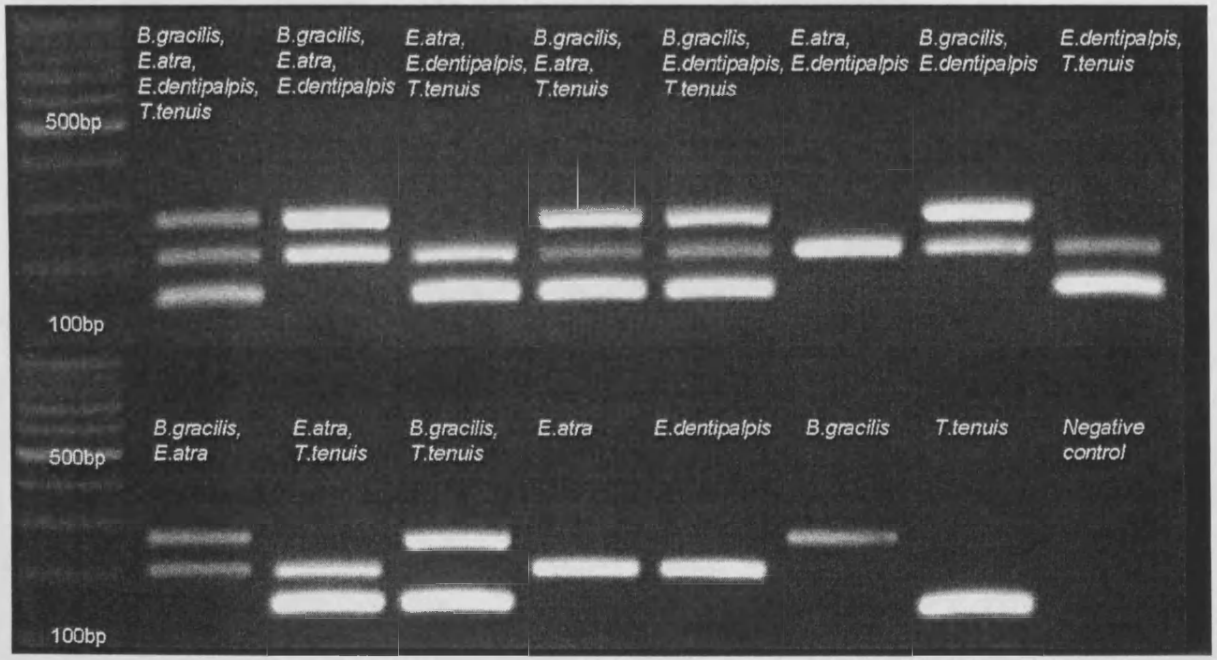


Fig. 6. Agarose gel showing diagnostic bands for each target species of juvenile spider following multiplex PCR. Every combination of target species was tested to ensure no 'dropouts' occurred. The reactions contained three primer pairs in each case; the *Erigone* spp. primer pair was designed to amplify the closely-related species *Erigone atra* and *E. dentipalpis* so these species are included separately and in combination.

OEDO MPLEX

Species	FWD (score)	REV (score)	SIZE (bp)	Mean Tm	Tm DIFF	µM each primer	2x MPLEX	BSA	Q	primer mix	DNA
Oedothorax retusus	270F (88)	571R (100)	300	62.2	5.6	0.25	5	0.1	1	1	1
Oedothorax fuscus	204F (87)	354R (99)	149	56.5	3	0.25					
Pachygnatha degeeri-M	277F (100)	399R (100)	123	63.1	3.8	0.25					

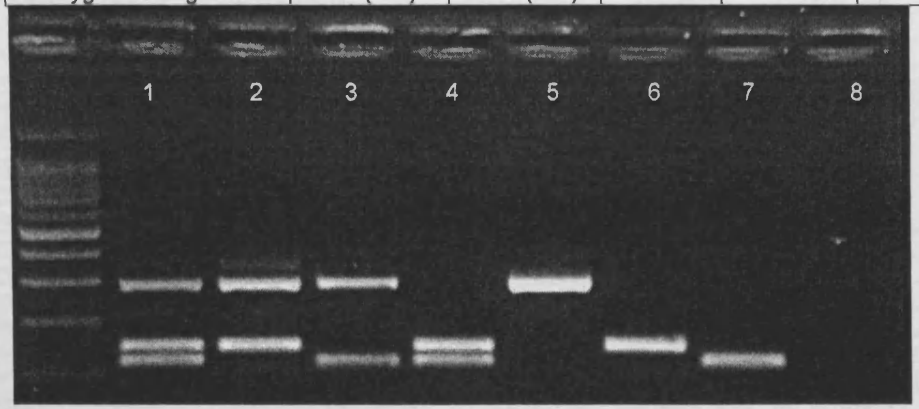


Fig. 6. Agarose gelimage showing optimised 'OEDO' multiplex. Lane 1 contains all three species, lane 2 *Oedothorax retusus* and *O. fuscus*, lane 3 *O. retusus* and *Pachygnatha degeeri* , lane 4 shows O. fuscus and P. degeeri, while lanes 5, 6 and 7 show O. retusus, O. fuscus and P. degeeri alone respectively. Lane 8 is a water-only negative control.

Predator species	Prey species	fragment size	replicate	Annealing temp (°C)	ambient temp(°C)	transformation	T50	SE	residual variance	slope	sig	AIC
Pterostichus melanrius	*Bathyphantes gracilis*	271	#1	62	16	log	15.67	1.49	3.7346 on 6	0.0061	**	26.55
Pterostichus melanrius	*Bathyphantes gracilis*	271	#2	62	16	log	13.73	1.33	2.0309 on 6	0.000634	***	23.98
Pterostichus melanrius	Bathyphantes gracilis	271	cum	62	16	log	24.65	1.47	3.8733 on 6	0.00474	**	25.82
Pterostichus melanrius	*Bathyphantes gracilis*	271	2x dilution	62	16	log	7.54	0.82	7.4174 on 5	0.115		28.04
Pterostichus melanrius	*Erigone* spp.	244	#1	62	16	na	3.76	5.44	4.9151 on 6	0.002	**	25.33
Pterostichus melanrius	*Erigone* spp.	244	#2	62	16	log	7.85	1.34	3.7428 on 6	0.0000772	***	24.47
Pterostichus melanrius	*Erigone* spp.	244	cum	60	16	log	12.87	1.32	4.6991 on 6	0.000285	***	26.73
Pterostichus melanrius	*Erigone* spp.	205	2x dilution	60	16	na, quas	13.70	3.45	6.9304 on 5	0.0219	*	NA
Pterostichus melanrius	*Tenuiphantes tenuis*	145	#1	61	16	log	30.28	1.65	2.6848 on 6	0.00836	**	26.97
Pterostichus melanrius	*Tenuiphantes tenuis*	145	#2	61	16	log	20.06	1.39	2.8534 on 6	0.001224	**	26.72
Pterostichus melanrius	*Tenuiphantes tenuis*	145	cum	61	16	log	43.66	1.48	4.3341 on 6	0.003084	**	24.03
Pterostichus melanrius	*Tenuiphantes tenuis*	145	2x dilution	61	16	log	7.31	0.60	12.320 on 5	0.0325	*	32.43
Pterostichus melanrius	*Pachygnatha degeeri*	318	#1	64	16	log, quas	13.09	1.18	7.8159 on 6	6.66E-06	***	NA
Pterostichus melanrius	*Pachygnatha degeeri*	318	#2	64	16	log	21.91	1.23	5.8389 on 6	0.000111	***	25.57
Pterostichus melanrius	*Pachygnatha degeeri*	123	#1	64	16	log, quas	18.49	1.33	15.919 on 6	3.52E-02	***	NA
Pterostichus melanrius	*Pachygnatha degeeri*	123	#2	64	16	log, quas	22.39	0.17	10.627 on 6	6.89E-05	***	NA
Pterostichus melanrius	Pachygnatha degeeri	123	#1	62	16	log, quas	22.97	0.20	10.554 on 6	0.000118	***	NA
Pterostichus melanrius	Pachygnatha degeeri	123	#2	62	16	log, quas	15.85	0.17	7.7046 on 6	1.64E-05	***	NA
Pterostichus madidus	*Tenuiphantes tenuis*	145	#1	61	16	log, quas	22.01	0.28	7.5487 on 6	0.0287	*	NA
Pterostichus madidus	*Tenuiphantes tenuis*	145	#2	61	16	log, quas	20.59	0.30	9.0451 on 6	0.0398	*	NA
Pterostichus madidus	*Tenuiphantes tenuis*	145	cum	61	16	na	35.67	9.09	8.6043 on 6	0.0194	*	30.09
Pterostichus madidus	*Tenuiphantes tenuis*	145	cum	61	16	log	26.22	0.30	3.2566 on 6	0.00547	**	24.75
Erigone atra	Isotoma anglicana	276	#1			log	48.48	0.10	1.1001 on 4	0.0267	*	9.01
Pardosa spp.	*Rhapalosiphum padi*	331	#1	58		na	3.57	0.53	13.569 on 6	4.59E-05	***	35.38
Anthocoris tomentosus	*Cacopsylla pyricola*	271	#1			na	21.46	1.19	4.1994 on 6	2.22E-09	***	16.89
Anthocoris tomentosus	*Cacopsylla pyricola*	188	#1			na	25.02	1.23	1.0671 on 6	1.34E-07	***	13.87
Tenuiphantes tenuis	*Lycoriella castanescens*	210	#1			na	35.11	5.78	8.4016 on 6	0.000954	***	26.04
Podisus maculiventris	*Lycoriella castanescens*	214	#1	54		log	45.83	0.44	9.630 on 5	0.000203	***	35.19
Coleomegilla maculata	*Lycoriella castanescens*	214	#1	54		na	6.87	0.80	2.3580 on 4	8.88E-09	***	21.45
Strateolaelaps miles	*Heterorhabditis megidis*	149	#1	60		log	4.64	0.20	2.551 on 3	0.000212	***	14.48
Strateolaelaps miles	*Phasmarhabditis hermaphrodita*	154	#1	62		log	5.88	0.19	2.6016 on 3	0.000215	***	14.41
Folsomia candida	*Steinernema feltiae*	200	#1	62		log	9.04	0.18	0.80725 on 4	5.76E-05	***	14.01
Predator species	Prey species	fragment size	replicate	Annealing temp (°C)	ambient temp(°C)	transformation	T50	SE	residual variance	slope	sig	AIC
Folsomia candida	*Heterorhabditis megidis*	149	#1	60		log	11.22	0.19	2.4346 on 4	4.86E-05	***	16.13
Homalodisca coagulata	*Chrysoperla carnea*	197	#1	50		na	12.07	2.26	17.939 on 3	0.000769	***	33.82