University of Kentucky
UKnowledge

Theses and Dissertations--Entomology

Entomology

2014

Exploring the Links Between Seasonal Variation and Spider Foraging

Thomas Edward Dantas Whitney
University of Kentucky, thomas.whitney@uky.edu

Recommended Citation

Dantas Whitney, Thomas Edward, "Exploring the Links Between Seasonal Variation and Spider Foraging" (2014). *Theses and Dissertations--Entomology*. 9.
https://uknowledge.uky.edu/entomology_etds/9

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student’s advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student’s thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Thomas Edward Dantas Whitney, Student

Dr. James D. Harwood, Major Professor

Dr. Charles W. Fox, Director of Graduate Studies

EXPLORING THE LINKS BETWEEN SEASONAL VARIATION AND SPIDER FORAGING

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Agriculture at the University of Kentucky

By
Thomas Edward Dantas Whitney
Lexington, Kentucky
Director: Dr. James D. Harwood, Associate Professor of Entomology
Lexington, Kentucky
2014

ABSTRACT OF THESIS

EXPLORING THE LINKS BETWEEN SEASONAL VARIATION AND SPIDER FORAGING

According to optimal foraging theory, generalist predators, such as spiders, are thought to feed indiscriminately on prey according to its availability, especially when food is scarce. In contrast, generalists can display selective feeding decisions under regimes of high prey abundance, but few studies have tracked changes in prey choice on a seasonal basis under open field conditions. Additionally, adaptations to surviving winter have been largely ignored in the research of foraging behavior. To elucidate this, I monitored prey availability and collected common forest-dwelling wolf spiders for molecular gut-content analysis, in parallel for 18 months, to assess the temporal changes occurring in spider preferences of common leaf litter prey. In addition, to determine if any physiological improvements to resisting low temperature mortality were affecting spider foraging, I also collected spiders monthly to track changes in spider supercooling points. The results revealed that spiders do exhibit selective feeding throughout the year, and appear to do so in a way that diversifies their diets. Also, despite low litter temperatures putting them in severe freezing risk, cold tolerance in these spiders remained unchanged throughout the winter, which suggests opportunity for growth during this uncompetitive period is paramount to accumulating survivorship-increasing, but also mobility-decreasing, cryoprotectants.

Keywords: Generalist predators, *Schizocosa ocreata*, *Schizocosa stridulans*, molecular gut-content analysis, supercooling point

Thomas Edward Dantas Whitney

April 11, 2014

EXPLORING THE LINKS BETWEEN SEASONAL VARIATION AND SPIDER FORAGING

By

Thomas Edward Dantas Whitney

Dr. James D. Harwood
Director of Thesis

Dr. Charles W. Fox
Director of Graduate Studies

April 11, 2014
Date

ACKNOWLEDGMENTS

I would like to thank Dr. James D. Harwood for his support and encouragement throughout my time working on this challenging project. His mentorship has greatly influenced my development as a young scientist. I also thank my committee members, Dr. John Obrycki and Dr. Lynne Rieske-Kinney, for their input during the integral steps in shaping my research. My collaborator, Dr. Benjamin Philip of Rivier University, conducted all supercooling assays and was integral in developing a major component of my research. I am especially grateful for Kacie Athey, who trained me and provided regular assistance in every step of molecular gut-content analysis and primer design. I would also like to thank Sarah Barney for her assistance with DNA extraction while my pipetting thumb was broken. I owe thanks to Victoria Pook and Adam Kesheimer for frequent spider-collection assistance, as well as Kacie Athey, Chris Strohm, Shelby Stedenfeld, Ryan Kepler, and Grady Kepler for their contributions in the field. Berea College Forest generously allowed me to use their research site. Most of all I’d like to thank all present and past members of the Harwood lab for their regular advice and for a gratifying experience. The National Science Foundation Graduate Research Fellowship Program and the University of Kentucky, Department of Entomology supported this research.

TABLE OF CONTENTS

LIST OF TABLES AND APPENDICES

LIST OF FIGURES

Chapter 1: Introduction and Literature Review

1.1 General Introduction

Leaf litter in temperate deciduous forests provides habitat for a wealth of invertebrate life. Almost 90% of primary production in a typical forest enters the detrital food web, where it becomes dead plant material and acts as the main source of energy for many of its organisms (Swift et al. 1979, Chen and Wise 1999). This supports numerous groups of detritivores, such as springtails (Collembola) and flies (Diptera) (Chen and Wise 1999), and in turn, an array of secondary arthropod consumers including ants, centipedes, predatory mites, pseudoscorpions, beetles, and spiders (Swift et al. 1979). An integral attribute of the litter layer, which encourages a vast number of invertebrates to coexist, is its high structural complexity (Figure 1.1). Fallen leaves and branches provide an assortment of crevasses and spaces within confined areas, which are ideal sites of refuge, egg laying, and feeding. Studies show that the augmentation of leaf litter, which increases detrital and structural resources, increases densities of fungivores (Chen and Wise 1999), and in turn, increases predator populations, such as carabids (Magura et al. 2004) and spiders (Rypstra and Marshall 2005, Oelbermann et al. 2008, Castro and Wise 2009, 2010). Therefore, a thick litter layer, common in Eastern deciduous forests, fosters a myriad of trophic interactions to exist.

In addition to these spatial factors, food web interactions are also driven by temporal factors. Characteristic of temperate forests is the regular transition between seasons across the year, involving a wide range of temperatures and other climatic conditions. Over evolutionary time, extant litter-dwelling invertebrates of these regions have developed phenological, physiological, and behavioral adaptations to survive during these environmental changes. These traits are diverse and are expressed differently according to the time of year, leading to a network of interactions that influences the trophic transfer of energy in cryptic ways. Collectively, these ecological interactions drive the formation of a dynamic food web structure, the intricacies of which we have only began to disentangle.

My study used a leaf litter system to investigate certain aspects of the decomposer food web on a seasonal basis. Specifically, this research monitored the foraging behavior of two generalist predators, the wolf spiders *Schizocosa ocreata* (Hentz) and *Schizocosa stridulans* (Stratton) (Araneae: Lycosidae) (Figure 1.2), to examine how seasonality affected patterns of their prey availability, and thus patterns of their prey acquisition and exploitation. Furthermore, the incidence of prey choice behavior during winter was compared to that of other seasons to determine how foraging tendencies in these winter-active predators complement, or possibly hinder, their ability to survive low temperatures. These facets of foraging ecology, especially those associated with winter, have been largely unexplored to date. I used a combination of field and molecular techniques to address these objectives, including the traditional sampling of invertebrate populations with pitfall trapping and the contemporary characterization of trophic linkages with PCR-based gut-content analysis. Ultimately, my work expanded on the basic foundation in our understanding about the interaction pathways between spiders and their litter-dwelling prey within the context of a seasonally dynamic environment.

Figure 1.1 The structurally complex leaf litter layer (Decmeber 14, 2011) of Berea College Forest in Madison County, Kentucky (USA).

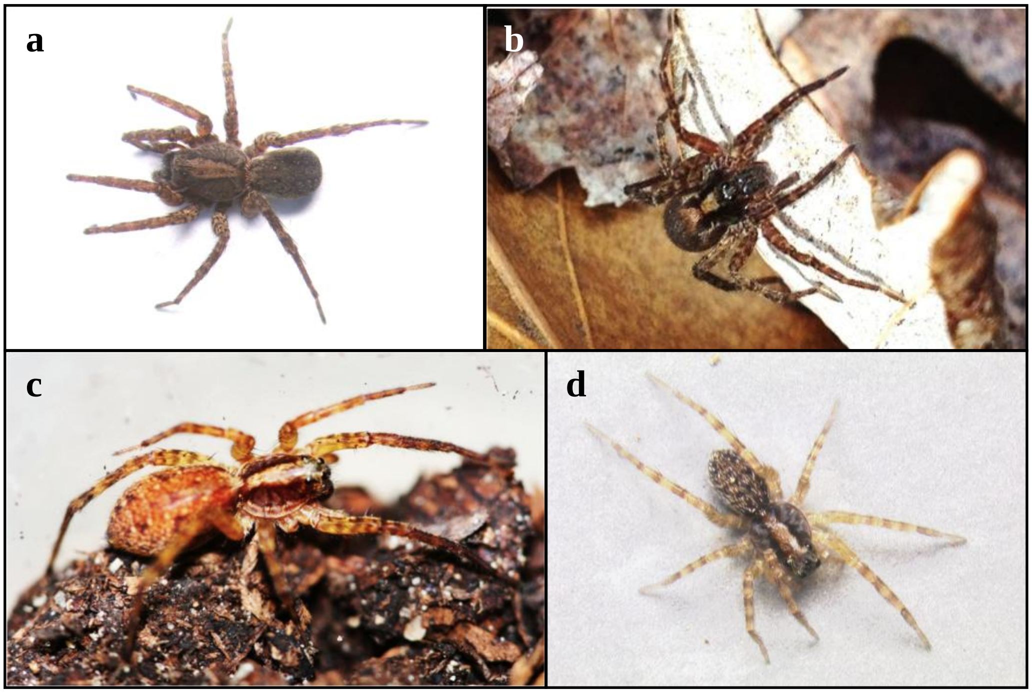

Figure 1.2 Wolf spiders (Araneae: Lycosidae), *Schizocosa ocreata* (Hentz) adult female (a) and juvenile (b) and *Schizocosa stridulans* (Stratton) adult female (c) and juvenile (d), collected from Berea College Forest in Madison County, Kentucky (USA).

1.2 Generalist Predators

Generalist predators are polyphagous, meaning they are able to consume many types of prey. This allows them to be more resistant to starvation during periods of sparse prey availability than specialist predators, which are limited to a narrower scope of food possibilities (Ehler 1977, Holt and Lawton 1994). Although polyphagy allows for a wider diet breadth, there are still many factors that reduce the possible food choices of a generalist predator, such as the size and activity patterns (i.e. susceptibility and availability) of the prey (Eubanks and Denno 2000). These parameters, however, do not limit the profitability of a prey item, but rather the potential for a prey item to be considered as food in the first place. This idea suggests that generalist predators are incapable of making truly selective feeding decisions, contending that the "choices" are made for them by encounter rates and vulnerability of prey to capture. In other words, they are assumed to simply forage opportunistically and indiscriminately, consuming the animals they have hunted or trapped with little selective influence (Stephens and Krebs

1986, Kamil et al. 1987, Galef 1996). Nevertheless, there is a growing body of evidence to suggest these predators can display selection based on other parameters, such as nutritional and energetic content of prey (Simpson and Raubenheimer 1993, Simpson et al 2004, Mayntz et al. 2005, Lee et al. 2008, Maklakov et al 2008, Mayntz et al. 2009, Pekar et al. 2010), the extent of which varies across taxa and environments. To gain insights into the dynamics of selective foraging behavior of generalist predators, two important components of the ecology and biology of prey must be addressed: availability and quality.

1.2.1 Prey availability

Most generalist invertebrate predators are food limited in terrestrial environments (Samu and Biro 1993, Bilde and Toft 1998, Harwood et al. 2003) and thus are largely thought to feed on prey arbitrarily when it is available. This argument is especially cogent in the case of sit-and-wait or trap-building predators, where rates of predator-prey encounters, and therefore rates of predation, strongly rely on prey activity-densities. In response to density shifts in the prey community, generalist predators are able to switch between consuming prey types (Riechert and Lawrence 1997), which maximizes consumption rate, and thus caloric intake. Although the presence of particularly profitable prey can influence feeding decisions when food is abundant, general food limitation likely forces generalists to feed primarily based on the availability of their prey rather than being too particular (Holt and Lawton 1994). Despite this logic, there are some empirically derived exceptions (Samu and Biro 1993, Harwood et al. 2004, Harwood et al. 2007, Schmidt et al. 2012). Our overall understanding of food availability and feeding responses of predators are limited and rely heavily on models and laboratory experiments (Tschanz et al. 2007). More studies in natural systems are needed to better understand how activity-densities of prey govern the feeding decisions of generalist predators.

1.2.3 Prey quality

The notion of prey quality arises frequently in the literature and is typically measured by how well predator fitness is sustained (Toft and Wise 1999). Unlike herbivores (Behmer 2009) and omnivores (Lee et al. 2008), with diets containing food items with a wide range in nutritional content, polyphagous predators are thought not to regulate nutrient intake, because animals as food items contain a more complete spectrum of nutrients and differ little among species (Stephens and Krebs 1986, Galef 1996). Rather, they are expected to optimize prey capture rate instead (Mayntz et al. 2005). However, prey animals do in fact vary greatly in quality among species based on nutrient composition, energy content, and toxicity, all of which greatly affect a predator's fitness (Marcussen et al. 1999, Toft and Wise 1999). Since various potential prey items differ in these three factors, a diverse diet is often times optimal for a predator, whereas a single prey diet may not suffice when they need to satisfy amino acid requirements, for example (Greenstone 1979, Toft and Wise 1999).

Despite the fact that mixed diets increase growth and fecundity in arthropod predators (Toft 1995, Harwood et al. 2009), it has been widely held that generalists lack the physiological and behavioral capabilities to select for prey that could provide these benefits (Harwood et al. 2009). More evidence is surfacing, however, to suggest the contrary (Jensen et al. 2011, 2012). For example, Mayntz et al. (2005) found that invertebrate predators can address protein and lipid needs by selective feeding. With abundant options under laboratory conditions, they observed selection at different stages of prey handling, pre- and post-capture, in three different predators. In addition to this, extreme selectivity in the form of partial consumption and wasteful killing in order to maximize feeding rate, has also been observed, but usually occurs only when prey are extremely abundant (Samu and Biro 1993). These conditions with ample options for food are rare in nature, presumably making selectivity for the highest quality prey energetically impractical for generalist predators under normal circumstances (Harwood et al. 2009). If and when prey does become plentiful under open field conditions, however, nutritional requirements may become a more important player in predator feeding decisions. Nevertheless, prey availability is thought to have the greatest influence in most situations.

1.2.4 Spiders

Spiders are extremely numerous in almost all terrestrial environments, especially in areas with heavy vegetation, like forests (Foelix 2011). Considered the most polyphagous of arthropod groups, they are dominant not only in numbers but also as carnivores (Toft and Wise 1999). Spiders constitute a major component of generalist predator guilds, situated at high and intermediate trophic levels (Moulder and Reichle 1972). Some can have omnivorous tendencies (Peterson et al. 2010, Schmidt et al. 2013), but their diets are mostly restricted to various arthropods (Nentwig 1986). Like other generalist predators, spiders are assumed to feed in close accordance to the availability of their arthropod prey (Nentwig 1982).

Nearly half of the world's spiders are cursorial, non-web spinners (Nentwig 1986, Foelix 2011). Major families of this group include Corinnidae, Salticidae, Gnaphosidae, Thomisidae, Ctenidae, Pisauridae, Clubionidae, and the widely studied Lycosidae. Grouped in the functional category of ground running, hunting spiders, lycosids, the wolf spiders, mostly employ a sit-and-pursue hunting mode (Uetz 1999). They wait to sense vibrations of nearby prey before ambushing for the kill, which is an energetically efficient hunting strategy (Foelix 2011) and allows lycosids to tolerate starvation well; some only need to consume one prey item per week to satisfy energy requirements (Wise 2004) and some species have been reported to live up to 200 days without feeding (Anderson 1974).

One of the most common prey items for lycosid spiders and other epigeal arthropod predators are springtails (Collembola). Given that they are widespread, abundant (Hopkin 1997), and mostly of excellent nutritional quality (Marcussen et al. 1999, Bilde et al. 2000), they are a primary prey resource for many spiders (Miyashita et al. 2003, Schmidt-Entling and Siegenthaler 2009). In fact, the addition of detritus as a supplemental resource for collembolans has shown to not only support, but also increase spider densities (Chen and Wise 1999, Harwood et al. 2003, Rypstra and Marshall 2005).

Collembola are exceptionally important prey for litter-dwelling spiders of forests, where herbivorous arthropods are not well represented in leaf litter microhabitats. Although they are abundant, the accessibility of Collembola and other potential lycosid prey is greatly affected by spatio-temporal aggregation patterns, which can have profound effects on foraging (Grear and Schmitz 2005, Lensing and Wise 2006, Shultz et al. 2006). Disentangling the changing food web of forest lycosids will offer a better understanding of the foraging tendencies in spiders and other generalist predators.

1.3 Seasonal Environmental Shifts

Invertebrate abundance and activity shift throughout the year in response to many factors, including climatic conditions. Warmer and wetter weather, to an extent, is generally more conducive to high faunal density and diversity than cooler and drier weather. During warm, prey rich periods, generalist predators are expected to have the luxury to exhibit more selection for the most profitable prey (Perry and Pianka 1997). In accordance with optimal foraging theory, generalists are also predicted to compensate during periods of low prey richness and availability by increasing their dietary diversity, feeding indiscriminately on individuals they encounter (Riechert and Harp 1987, Begg et al. 2003). Previously, these hypotheses have not been tested together as part of a comprehensive seasonal examination of foraging behavior in spiders. How the strength in trophic linkages change between regimes of low and high prey availability remains unclear, especially when compounded with physiological adaptations associated with winter-activity.

Low temperatures present a difficult challenge to spider survival. There are five different spider life cycles, which determine how these animals cope with winter: (1) eurychronous spiders take multiple years to mature, so they overwinter in various life stages; (2) diplochronous spiders reproduce twice a year, overwintering as adults; a subset of (3) stenochronous spiders overwinter as immatures and reproduce in the warm months following; a subset of (4) stenochronous spiders lay eggs in autumn and overwinter as spiderlings; and the winter-reproductive species, another subset of (5) stenochronous spiders, reproduce during the winter (Aitchison 1984b, Catley 1992, Foelix 2011). For the purposes of this thesis, winter-activity will be broadly defined after Aitchison (1987) as locomotory movement at temperatures of 2 ˚C or lower. Linyphiidae, Clubionidae, Thomisidae, and Lycosidae are among the families with species commonly deemed to be winter-active (Bayram and Luff 1993b, Foelix 1996, Vanin and Turchetto 2007). Although these life history categories have been distinguished, the significance of winter-activity to spider survival and foraging behavior is not fully understood.

1.3.1 Overwintering strategies

Many arthropods and about 85% of spiders are effectively dormant over most of the winter season, remaining relatively passive in the well-insulated soil or leaf litter (Gunnarsson 1985, Foelix 2011). Mortality is surprisingly low due to reduced metabolic rate and antifreezing agents in their hemolymph. Winter-inactive spiders generally have increased glycerol content in their body fluid during the cold months, which prevents formation of ice crystals (Husby and Zachariassen 1980) that cause high levels of mortality. The few spider species that remain active during this time reduce their metabolism, but not to the extent of those in diapause. For this reason, winter-active spiders require better circulatory flow to sustain mobility, but glycerol is not ideal as an antifreeze agent, because it thickens the hemolymph (Husby and Zachariassen 1980). Instead, some can possess certain proteins that cause thermal hysteresis of the body fluid’s freezing-melting point (Zachariassen 1985, Catley 1992), which allows them to have a relatively high metabolism without circulatory complications associated with viscous hemolymph.

In temperate environments with harsh winter conditions, the accumulation of such cryoprotectants are especially important to counteract any possible freezing risk associated with winter-active foraging. Prey consumption can increase the probability of freezing, because food in the alimentary canal is a common source of ice nucleating agents, which are prerequisite to the beginning of spontaneous ice crystallization (Salt 1961). In general, fewer ice nucleators and/or more cryoprotectant chemical accumulation can drastically reduce the chances of ice crystal formation in the hemolymph, which fatally damages internal structures in most invertebrates (Sinclair et al. 2003). The balance between food intake and antifreeze production in winter-active arthropods requires more research to better develop the foundational knowledge for the biology of these animals.

1.3.2 Low temperature feeding

Like spiders, the majority of the prey community usually takes refuge in a dormant state over winter. Although winter-active spiders are able to feed on members of this hibernating group (Juen et al. 2003), encounter rates are likely low due to the passive state of the prey and the reduced foraging activity of the predators. Therefore, the bulk of their food in low temperatures comes from other winter-active invertebrates, which comprise a narrower, less available diet breadth for spiders relative to that of the warm season. These spiders respond to the lack of resources by consuming little and using less. Aitchison (1984a) found that at 0 ˚C, winter-active spiders were capable of locomotion, but typically fed infrequently. Despite this, they have been shown to not just survive, but also sustain steady, slow growth (Aitchison 1984a), which can provide reproductive benefits in spring (Gunnarsson 1988).

With a simplified food web during the winter, there are fewer prey options for spiders. Detritivores become even more prominent during this time, because living plant tissue for herbivory is scant. At the soil surface, the most abundant invertebrates are thin-cuticled Collembola, primarily of the families Entomobryidae and Tomoceridae (Aitchison 1984a). These cold-tolerant Collembola can feed down to -2.5 ˚C and represent the majority of prey items accessible to winter-active lycosids and linyphiids (Aitchison 1984a). During cold temperatures, however, Collembola form large colonies that move in a highly synchronized manner, which results in varying hunting success for spiders (Block and Zettel 2003). Aitchison (1984a) observed spiders to waste little from prey corpses below temperatures of 5 ˚C, efficiently extracting 99% of the mass from captured food. This contrasts with similar species exhibiting partial consumption and wasteful killing during warm months when prey is more available (Samu and Biro 1993), suggesting a definite difference in foraging exists according to the time of year. Given these factors that affect spider feeding, I sought to extend our knowledge across a longer temporal scale, tracking the strength and changes in spider predation along different trophic pathways over the year.

1.4 Research Objectives

The principal objectives of this research were as follows:

1. Determine the degree to which seasonal changes in prey availability dictates foraging behavior in *Schizocosa* over time.
2. Explore the extent of *Schizocosa* cold tolerance and examine its relationship with winter prey consumption.

Chapter 2: The effect of prey availability on the seasonal predation patterns of forest-dwelling spiders

2.1 Summary

As generalist predators, spiders are thought to feed opportunistically during times of low prey availability. This is complicated, however, by the inevitable temporal variability in invertebrate populations, which provides spiders varying access to prey over their life cycle. The primary objective of this study was to identify seasonal spider predation patterns in response to seasonal variation in availability of the three common potential prey groups: springtails (Collembola), flies (Diptera), and small crickets (Ensifera). Within a temperate deciduous forest ecosystem, prey availability was monitored and, in parallel, the dominant epigeal spiders from the genus *Schizocosa* (Araneae: Lycosidae) were collected for molecular gut-content analysis to track temporal shifts in trophic strength. Prey availability and predation of three common groups (Collembola, Diptera, and Ensifera) were correlated using the linear food selection index to quantify temporal differences in spider preference. Despite variation in prey populations throughout the year, spiders fed independently of Collembola availability and unexpectedly foraged for them selectively during the first winter when their populations were lowest. Therefore, temperature, rather than prey availability, was a more accurate predictor of Collembola predation frequency. In addition, spiders fed on Diptera selectively as well, seemingly in a way that supplemented their diet in balance with collembolans. Ensifera seldom were consumed regardless of availability. My findings suggest that spiders are capable of selective feeding decisions, which indicates that foraging is not dependent on the frequency at which prey is available during all parts of the year. Moreover, these spiders seemed to diversify their diet, which is associated with increased fitness. Molecular detection of trophic interactions in this forest system provided insight into the role of the dominant *Schizocosa* spiders in a complex leaf-litter food web, especially during the winter where there is a paucity of information concerning predator-prey interactions.

2.2 Introduction

Foraging can be reduced to the repetition of three basic sequential events in most models: a searching period for food, an encounter with a potential food resource, and a decision of whether to consume it or neglect it for continued searching (Stephens and Krebs 1986). Optimal foraging theory (OFT), an application of economics to biology where energy is the primary currency, is often used to make predictions about how organisms will feed. This theory predicts that organisms will strive to optimize their resource acquisition per unit time, minimizing the energy expended: gained ratio of each foraging event (Macarthur and Pianka 1966). This is generally achieved by decreasing searching time (duration between encounters) and handling time (duration required to extract energy) of a food resource, which increases the profitability (as measured by net energy gain) of each meal. However, there are several critics of OFT as a fully inclusive model for predicting foraging behavior (Perry and Pianka 1997). For example, some point out that OFT only recognizes energy as the main factor that drives feeding decisions, and incorrectly makes the assumption that other factors, such as nutritional content, are equal for all potential food (Slansky and Scriber 1985, Stephens and Krebs 1986, Galef 1996). Despite this, there are still tenants of OFT where empiricists and theorists come to consensus. For example, considered one of the most robust theorems of OFT is the prediction that during times of relative food scarcity, individuals cannot afford to be as selective about what they eat compared to during times of relative food abundance (Perry and Pianka 1997). This is thought to occur because as food densities decrease in an environment, searching time, and therefore expended energy, increases between food encounters. More searching time implies there are fewer overall encounters, which results in fewer opportunities to make decisions. Thus, it is logical to assume maximization of consumption rate is the optimal foraging strategy in this case. In contrast, if positive net energy intake is easily obtained when prey is abundant and searching time between encounters is reduced, it then may be optimal for an organism to be more selective and increase the profitability of a meal in other ways (e.g. select for certain nutrients) (Williams 1987). This introduces an important, but understudied component of foraging theory: seasonal changes in the accessibility of prey and its effects on higher trophic levels. Despite some empirical support for the hypothesis that organisms forage differently based on the abundance of food (reviewed by Gray 1987), the overall number of studies is limited and more investigation is required.

I examined seasonal foraging tendencies of generalist predators, which require certain considerations for making predictions stemming from OFT (Symondson et al. 2002). First, the mode of resource acquisition affects how organisms are predicted to forage. In the case of predators, they can be broadly categorized as either sit-and-wait hunters or active hunters, although there can be overlap. Those that employ a sit-and-wait strategy are presumably quite limited by the frequency of prey occurring in their hunting area or trap (Schmitz 2007). In some cases, sessile prey may not be available to these predators at all, but in the case of active hunters, there are fewer limitations to diet breadth, because they encounter active and torpid prey at a more equal rate (Scharf et al. 2006). In this regard, active hunting is advantageous, but when comparing the energy inputs, a sit-and-wait hunting style has considerably less initial investment, which can be advantageous as well. Second, the polyphagous nature of generalist predators allows them to consume a wider breadth of prey types. In contrast to specialist diets, which are primarily limited by the abundance of their narrow suite of potential food resources (Symondson et al. 2002), generalist diets can further complicate optimal foraging predictions. This is due to an additional host of factors that can affect their feeding decisions, such as size, activity, and nutritional stoichiometry of both the predator and prey. Despite the ability to feed on a wide range of prey, most predict that generalist predators lack the capacity to make selective feeding decisions, while others have experimentally revealed evidence of the contrary (Tschanz et al. 2007, Fantinou et al. 2009). Most studies that have displayed selective foraging in generalists, however, are laboratory-based and expose the predator to an artificial level of prey abundance. These are unnatural scenarios in most cases, because not only are natural prey populations highly variable spatially and temporally (Kato et al. 2003, Venner and Casas 2005), but much research has suggested that generalist predators are overall very food limited in their environments (Wise 1993, Bilde and Toft 1998). Because of this and their ability to switch between prey (Murdoch 1969), the consensus is that the most optimal foraging strategy for generalist predators is to simply maximize prey capture rate rather than expressing preferential prey choices, which causes them to be largely dependent on the frequency at which their prey is available to them (Stephens and Krebs 1986, Galef 1996). This is expected to be especially true when exposed to low levels of prey densities when the searching time, and thus the energy input per meal, is increased.

Ubiquitous in almost every terrestrial environment (Foelix 2011), spiders are abundant generalist predators. They have been shown to significantly affect prey populations in natural (e.g. Finke and Denno 2004) and disturbed systems (e.g. Riechert and Bishop 1990). However, the way in which spiders forage in response to varying prey availability is unclear, but likely varies across different families and functional groups. For instance, spiders can most broadly be categorized as web spinning, sit-and-wait hunters or wandering, active hunters (Uetz 1999), which exposes different species to varying densities and types of prey. As a group, however, spiders are relatively energy efficient arthropods, as their basal metabolic rate is lower than other invertebrates of similar size (Anderson 1970, Greenstone and Bennett 1980, Anderson and Prestwich 1982). This suggests that spiders have experienced food shortages often throughout their evolutionary history (Wise 1993). As a result, some species can meet energy requirements on only one prey item per week (Wise 2004), but in order to grow and maximize reproductive fitness, more energy and macronutrients from food are needed. These distinctive characteristics of spiders may promote a unique optimal foraging strategy; some suggest spiders are very limited by prey availability (Nentwig 1982), while others argue they are quite selective (Wise 2006). When given a choice, some laboratory studies indicate spiders can select for certain nutrients (Jackson et al. 2005, Wilder and Rypstra 2010). However, they are assumed to forage similarly to other generalist arthropod predators in food limited field conditions, consuming prey opportunistically in a frequency-dependent fashion to varying degrees, depending on prey availability (Nentwig 1982, Riechert 1991).

In this study, I examined the temporal feeding patterns of two species of *Schizocosa* (Lycosidae: Araneae), *S. ocreata* and *S. stridulans*. These species are vastly abundant in hardwood forest leaf litter and are widespread across Eastern North America (Stratton 1991). Furthermore, they are good candidates for studying seasonal changes in foraging behavior, because they are active throughout the entire year. Seasonal foraging dynamics of spiders and other arthropod predators remain largely overlooked in the literature, especially during the winter, when prey populations dwindle. For a winter-active predator, exposed to a scarce food supply while needing to meet energy requirements to sustain activity, an opportunistic, non-selective foraging strategy to maximize food intake per unit time seems ideal. However, these *Schizocosa* species overwinter as juveniles, maintaining a slow and steady growth rate until spring and summer when they mature and become reproductively active (Klawinski 1996). Growth requirements during winter, compared to reproductive requirements during more favorable parts of the year, may promote unexpected shifts in prey preference if the ability to selectively forage exists in these spiders. In addition, *Schizocosa* are ground running spiders, but should not exclusively be considered active hunters. Lycosids typically remain still when foraging, sensing vibrations of unsuspecting invertebrates through the litter substrate to locate a meal (Foelix 2011). Thus, many classify wolf spiders as ‘sit-and-pursue’ hunters (Uetz 1999), which is a hunting style that has not been extensively investigated with regards to prey preference.

Physically observing predation in the field, especially in wolf spiders that masticate their prey and digest extra orally (Wilder 2011), is impractical for large-scale foraging studies. The recent implementation of molecular methods for the detection of trophic interactions, however, has vastly improved our understanding of food webs and is now a commonplace technique (Symondson 2002). In the current study, I used molecular techniques to characterize the trophic interactions between *Schizocosa* spiders and the common prey groups available to them over the span of a year and a half in a Kentucky forest. This was done to monitor any foraging shifts that may be occurring in response to seasonal variation in prey populations. The goal was to test the hypotheses that these generalist predators are (1) largely restricted in their feeding according to the availability of their prey, and (2) feed more selectively during times of overall high prey availability and feed more indiscriminately during times of overall low prey availability, such as during the winter.

2.3 Materials and Methods

2.3.1 Field site

All field research was conducted at Berea College Forest in Madison County, Kentucky, USA (37˚34’22”N, 64˚13’11”W, elevation ~ 268 m), within a temperate deciduous forest, consisting mainly of oak and maple with scattered pine (Chen and Wise 1999). Within two sites of similar forest structure (established ~ 1 km apart), prey availability was monitored and spiders were routinely collected for molecular gut-content analysis between October 2011 and March 2013, encompassing two full winters (Figure 2.1).

2.3.2 Monitoring of abiotic factors

Leaf litter has low thermal conductivity and thus, reduces variation in temperature compared to air (Edgar and Loenen 1974, Kraus and Morse 2005). Given this, three HOBO Pro v2 data loggers (Onset, Cape Cod, MA, USA), oriented >100 m apart, were positioned 1 cm above the soil surface to monitor temperatures and relative humidity experienced by spiders in their epigeal leaf litter habitat (Figure 2.2). Measurements were recorded hourly throughout the study period. Daily and monthly mean temperatures were calculated for analyses.

Figure 2.1 Seasonal progressions in Berea College Forest field site, located in Madison County, Kentucky (USA) between autumn 2011 and spring 2012. To be active throughout the year in this temperate climate, spiders must cope with a changing environment.

Figure 2.2 Data loggers, positioned with the sensor ~1 cm above the soil, recorded temperature hourly between October 2011 and March 2013 at Berea College Forest in Madison County, Kentucky (USA).

Figure 2.3 Pitfall trap with Styrofoam rain guard, used to measure activity-densities of arthropods between October 2011 and March 2013 at Berea College Forest in Madison County, Kentucky (USA).

2.3.3 Assessment of prey availability

Pitfall traps (n = 32) containing ethylene glycol were used to monitor the surface-active invertebrate community in the leaf litter (Figure 2.3). Throughout the study duration, traps were collected every 6 – 12 days, and at least one of these sample sets was sorted for prey availability data every month. These samples offered seasonal activity-densities of invertebrates, which were used to measure prey availability for spiders. Despite not reflecting absolute densities, this metric is advantageous for studying predator-prey dynamics (Nentwig 1982). Invertebrates were identified to the lowest taxonomic group possible and were either deemed as potential prey (Appendix A) or non-prey (Appendix B) for spiders based on previous records and size criteria; non-web building spiders more readily consume prey that have a $\leq$ 1 prey/predator body size ratio (Nentwig and Wissel 1986, Moya-Larano and Wise 2007). This involved excluding particularly large individuals (e.g. crickets weighing > 150% of predator body size) from the potential prey category. Life stages of certain holometabolous groups (e.g. Coleoptera) were also excluded as potential prey, because evidence suggests that lycosids prefer soft-bodied arthropods, such as beetle larvae to adults (Oberg et al. 2011). Prey known to be toxic to spiders were also excluded, such as hypogastrurid Collembola (Bitzer et al. 2004).

2.3.4 DNA extraction and sequencing of spider prey

Based on rank abundance from pitfall data, the most common non-intraguild prey groups for spiders were Collembola, Diptera, and orthopterans from the suborder Ensifera, which were designated as the target prey to be tested during gut-content analysis. Individuals from these target prey groups, along with individuals for other potential prey groups not to be directly tested, were collected for sequencing from pitfall samples post-mortem and live from the litter (in separate 1.5 µL microcentrifuge tubes with 95% EtOH at -20 ˚C). Besides developing primers for target prey, the intent was to create a library of forest prey DNA sequences for cross-reactivity tests. Those arthropods used from pitfall samples for species identification were washed thoroughly with DI H_20 and 95% EtOH before extraction to limit possible contaminant DNA.

Total DNA was extracted from arthropods using Qiagen DNEasy® Tissue Extraction Kit (Qiagen Inc., Chatsworth, CA, USA) following the animal tissue protocol outlined by the manufacturer. For all arthropods, both to be used for primer design and cross-reactivity trials, DNA from leg tissue was extracted when possible, so as to avoid amplifying DNA from gut-contents. Whole body extractions were necessary for particularly small taxa, however, such as Collembola and most Diptera. The resulting 200 µl extractions were stored at -20 ˚C until polymerase chain reaction (PCR).

To gather sequences for species identification and primer design, the detailed procedures in Chapman et al. (2013) were followed. In summary, a portion of the cytochrome oxidase subunit I (COI) gene was sequenced from a minimum of five individuals per species using the general arthropod primers LCO-1490 and HCO-2198 (Folmer et al. 1994). Each reaction of 50 µL contained 1x Takara buffer (Takara Bio Inc., Shiga, Japan), 0.2 mM dNTPs, 0.2 µM of each primer, 0.625 U Takara *Ex Taq*™ and 2 µL of template DNA. BioRad PTC-200 and C1000 thermal cyclers (Bio-Rad Laboratories, Hercules, CA, USA) were used for PCR reactions under the following protocol: 94 ˚C for 1 min, followed by 50 cycles of 94 ˚C for 50 s, 40 ˚C for 45 s, 72 ˚C for 45 s and a final extension of 72 ˚C for 5 min. Electrophoresis of 10 µL of each PCR product was later conducted to determine success of DNA amplification using 2% Seakem agarose (Lonza, Rockland, ME, USA) stained with 1x GelRed™ nucleic acid stain (Biotium, CA, USA). Positive PCR products were sequenced by Advanced Genetic Technologies Center at the University of Kentucky, which were subsequently used to conduct *BLASTN* searches (Karlin and Altschul 1990) of GenBank and the Barcode of Life Database (BOLD) (Ratnasingham and Hebert 2007) for previously submitted sequences that significantly matched the organisms of interest. A significant match in GenBank and Barcode of Life Database was considered to be ≥ 97% max identity (percent similarity between the query and subject sequences) (after Hebert et al. 2003).

2.3.5 Collembola and Diptera primers and cycling optimization

Order-specific primers from the literature were used to detect the DNA of Collembola and Diptera, the two most numerous and active prey groups across the year, within the guts of *S. ocreata* and *S. stridulans*. For Collembola, primer pairs targeting the 18s rDNA gene were used. Chapman et al. (2013) modified one of the forward primers from Kuusk and Agustí (2008), Col4F, to control for cross-reactivity to some linyphiid spiders. Within the system of the current study, however, these modified group-specific primers did not function consistently well, so Collembola primers from Sint et al. (2012) were utilized. Here, the authors instead modified the reverse primer from Kuusk and Agustí (2008), Col5R, using sequences from GenBank to combine with one of the original forward primers, Col3F (Table 2.1). This primer pair worked well in this system. PCR cycling protocol for 12.5 µL reactions with Takara reagents (as above) and 1.5 µL of template DNA was optimized as follows: 95 ˚C for 1 min, followed by 35 cycles of 94 ˚C for 30 s, 61.2 ˚C for 90 s, and 72 ˚C for 60 s. For Diptera, primer pairs targeting the 18S gene were used after Eitzinger et al. (2013). PCR cycling protocol for 12.5 µL reactions with Takara reagents (as above) and 2 µL of template DNA was optimized as follows: 95 ˚C for 1 min, followed by 40 cycles of 94 ˚C for 45 s, 60 ˚C for 45 s, and 72 ˚C for 45 s. To test specificity, primers were tested against 93 non-target species for cross reactivity (Appendix C). Twenty-five of the non-targets came from the Berea College Forest field site.

2.3.6 Design of Ensifera species primers

COI primers of the most common species of Orthoptera were designed to determine the predation frequency on this group of relatively large and periodically available prey in *S. ocreata and S. stridulans*. Of the three distinct species regularly found in the field, searches in GenBank and BOLD yielded two significant matches within family Gryllidae: *Gryllus veletis* (Alexander and Bigelow) (97% - 99.8% max identity) and *Allonemobius maculatus* (Blatchley) (98% - 100%). No significant matches were found for the third species, but morphological characteristics and the 92% max identity match to similar sequences from GenBank and BOLD confirmed it was a species of *Ceuthophilus* Scudder (Orthoptera: Rhaphidophoridae). The lowest taxonomical commonality of these three orthopterans is the suborder Ensifera, containing the crickets and katydids, which will be the term used hereafter when referring to these species as a whole. Forward and reverse sequences of individuals were assembled using Geneious (Kearse et al. 2012), and additional editing and multiple sequence alignments were conducted using Bioedit Sequence Alignment Editor© (Carlsbad, CA, USA) and MUSCLE (©European Bioinformatics Institute, 2011; available online at http://www.ebi.ac.uk/Tools/msa/muscle/). Sequences of closely related species from GenBank were also used to determine similarity. Primers were designed for all three species of crickets and their parameters were tested using Primer3 (Rozen and Skaletsky 1998). The primer sets had amplicon sizes between 150 and 300 bp (Table 2.1). Upon receiving the primers, temperature gradients were run to determine optimal melting temperature. A uniform PCR cycling protocol for 12.5 µL reactions with Takara reagents (as above) and 1 µL of template DNA was optimized for all three Ensifera species as follows: 95 ˚C for 1 min, followed by 50 cycles of 94 ˚C for 45 s, 64 ˚C for 45 s, 72 ˚C for 45 s with no extension time. As with the Collembola and Diptera primers from the literature, these designed Ensifera primers were also screened against non-target species for cross reactivity (Appendix C).

Table 2.1 Targeted taxa/groups, primer names and sequences, size of amplicon, and source of design for the detection of prey taxa within the guts of *Schizocosa* spiders. All primer pairs were used in singleplex PCR assays.

Target group	**Primer names and sequences 5'-3'**	**Size (bp)**	**Source**
Collembola	Col3F: GGACGATYTTRTTRGTTCGT Col-gen-A246: TTTCACCTCTAACGTCGCAG	228	Sint et al. 2012
Diptera	DIPS16: CACTTGCTTCTTAAATrGACAAATT DIPA17: TTyATGTGAACAGTTTCAGTyCA	198	Eitzinger et al. 2013
Gryllus veletis	Gvel71F: CAACCAGGTTATTTAATTGGAGAC Gvel316R: TGTTCCTGCACCATTTTCAA	246	Whitney & Harwood unpublished
Allonemobius maculatus	Amac54F: AACTGAATTAGGACAACCAGGG Amac268R: CTGTACCTGCTCCATTTTCTACTAA	215	Whitney & Harwood unpublished
Ceuthophilus sp.	Ceuth275F: CACATTATTACTAGCAAGCAGCCTT Ceuth453R: GATTGTAGTAATAAAATTTACAGCACCA	178	Whitney & Harwood unpublished

2.3.7 Predation frequency of spiders on common prey

Every 6-12 days when possible, 10 to 40 spiders were collected during the 18-month study duration on plots adjacent to the pitfall traps so as to not interfere with the prey availability survey. Capture success was highly dependent on weather, resulting in monthly fluctuation of sample size. Like all lycosids, the eyes of *S. ocreata* and *S. stridulans* reflect light, so collections were done at night using headlamps to easily locate individuals active on the litter substrate. Spiders were removed from the litter using an aspirator, placed in separate 1.5 mL microcentrifuge tubes filled with 95% EtOH, and preserved at -20 ˚C upon return to the laboratory until DNA extraction.

DNA from *S. ocreata* and *S. stridulans* were extracted according to the protocol outlined above, with minor modifications. Here, whole bodies of the spiders were first crushed to release prey DNA from within their alimentary canal for extraction. For large adult spiders, legs were removed before extraction to increase the prey: predator DNA ratio, but coxae were left intact, because some digestive filaments extend into these structures (Foelix 2011). The 200 µL extractions were stored at -20 °C until PCR.

Spiders were screened for three main prey groups using PCR: springtails (Collembola), flies (Diptera) and crickets (Orthoptera: Ensifera), represented by *G. veletis*, *A. maculatus* and an undetermined *Ceuthophilus* species, all using the primer pairs (Table 2.1) and PCR cycling protocols described above.

2.3.8 Feeding trials and DNA detection

To delineate the effect that temperature has on DNA decay rates in this system, two groups of *S. ocreata* were collected for feeding trials: one group (n = 93) in August 2012 for the high temperature experiment and the other group (n = 129) in February 2013 for the low temperature experiment. By collecting spiders for the high and low temperature trials during the summer and winter, respectively, the need and possible complications of an acclimation period were avoided. For the high temperature experiment, spiders were maintained at 25 ˚C under a 15L:9D regime, simulating a common summer day in central Kentucky. Each spider was fed a single *Sinella curviseta*

Brook (Collembola: Entomobryidae) and then starved for 7 days before the experiment. After the starvation period, all spiders were fed a single *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) at room temperature (~ 22.5 ˚C) and were returned back to the growth chamber after the feeding period. Groups of spiders (n = 10) were subsequently preserved at 0, 2, 4, 8, 12, 16, 24, 36, and 48 hours in 95% EtOH, previously chilled at -20 ˚C, which was discovered to ensure a rapid death and prevents regurgitation of gut contents. A group of control spiders were not fed and were preserved before the feeding period.

For the low temperature experiment, spiders were maintained at 5 ˚C under the same 15L:9D regime to isolate temperature as the single independent variable. As before, spiders were fed a single *S. curviseta* and then starved for 7 days before the experiment. After the starvation period, all spiders were fed a single *D. melanogaster* at room temperature and were returned back to the growth chamber after the feeding period. Spiders (n = 10) were subsequently preserved at 0, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, and 192 hours in chilled 95% EtOH. As above, a group of control spiders were not fed and were preserved before the feeding period.

2.3.9 Statistical analysis

To determine which factors influenced predation of Collembola, Diptera, and Ensifera in these spiders, the predation frequency data obtained from PCR was analyzed using logistic regression with a binomial distribution and logit link. Any possible overdispersion was corrected for during the analysis. For each target prey group, the presence or absence of prey DNA from gut-content analysis was the dependent variable in the three separate analyses. Each analysis tested if consumption of prey was affected by the availability of the target prey, the availability of alternative potential prey, mean daily temperature, species (*S. ocreata* and *S. stridulans*), and life stage (immature and adult) in multiple logistic regressions. This analysis was conducted in JMP 10 (SAS$^{©}$ Institute 2012).

To examine the hypothesis that spiders feed in close accordance with the availability of their food, monthly predation frequencies were compared with monthly prey availability using the linear food selection index (*L*) (Strauss 1979). This index is the simple unweighted difference in proportions

$$L = r_i - p_i$$

where r_i is the relative activity-density of prey item *i* compared to other possible prey options occurring in the environment, and p_i is the relative abundance of prey item *i* in the predator's diet. The outcome is a result between -1 and +1, where a value of zero indicates random, frequency-dependent feeding, and values away from zero indicate selective feeding: positive results suggest preference for prey *i* and negative results suggest avoidance of prey *i*. Extreme *L* values are only observable when prey is rare but consumed exclusively, or when prey is abundant but ignored completely. According to the hypothesis of the current research, an *L* value of zero was expected for all prey, especially during colder, less prey-rich months.

2.4 Results

2.4.1 Monitoring of abiotic factors

Characteristic of a temperate forest, temperatures at the study site varied greatly over the year (Figure 2.4). Both winters during this study, however, were considered to be relatively mild compared to historic norms, averaging 4.93 ˚C and 4.22 ˚C from December to February each year, respectively. This was especially apparent in the first winter, because daily high temperatures were greater than normal and spring arrived early; the mean temperature in March 2012 was 14.47 ˚C, compared to 4.48 ˚C in March 2013. Despite this, leaf litter temperatures during the winters were frequently below zero. In contrast, the only summer examined was hot, surpassing 30 ˚C several times. Although relative humidity was also recorded, it was ignored for analyses, because significant differences between seasons were not detected.

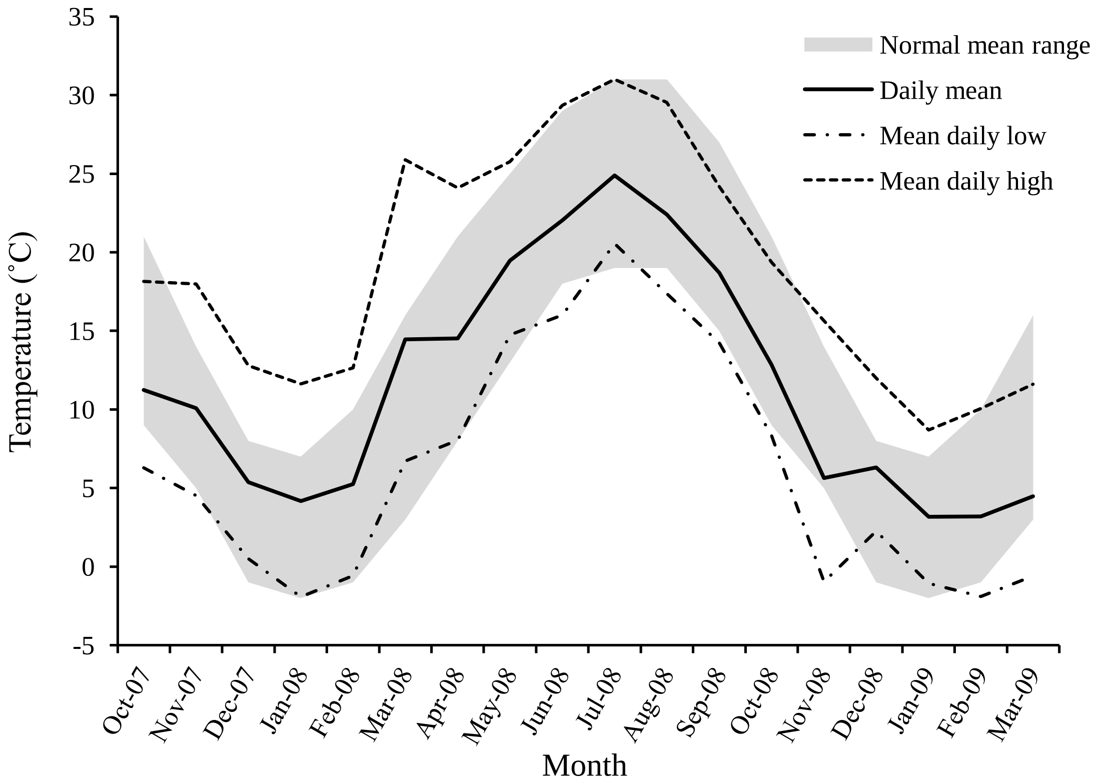

Figure 2.4 Monthly averages of daily mean, high, and low temperatures recorded by data loggers placed just above soil. Shaded region shows normal mean air temperature range each month in Berea, KY according to the National Weather Service (www.weather.gov).

2.4.2 Assessment of prey availability

The major potential prey of *Schizocosa* consisted of 56% Collembola, 17% Diptera, and 6% Ensifera (Figure 2.5). A large portion of the remaining 21% of potential prey included small Araneae and Coleoptera, but several other less common arthropods were also available for spiders, such as members from Blattodea, Hemiptera, Psocoptera, and Lepidoptera (Appendix A). Collembola were the most available prey group, detected at an overall mean 1.28 individuals/trap/day (Figure 2.5). They were also the most abundant prey group during every season in the study, except for winter 2011-2012. The difference between the two winters is worth noting: activity-densities of dipterans were highest during the first winter, whereas activity-densities of collembolans were highest during the second winter (Figure 2.6). The marked difference in Diptera availability between winters can chiefly be explained by a strong presence of wingless crane flies, from the genus *Trichocera* (Diptera: Trichoceridae), in December 2011 through February 2012, but not in December 2012 through February 2013. Apart from the first winter, Diptera consistently represented the second most available target prey (mean of 0.4/trap/day), and Ensifera consistently represented the least available target prey (mean of 0.13/trap/day) across seasons (Figure 2.5). All other potential prey were collected in pitfall traps at an overall mean of 0.48 individuals/trap/day.

Total prey availability was positively associated with temperature ($R^2 = 0.56$, $F_{1, 17} = 20.29$, $P = 0.0004$) (Figure 2.7a), resulting in the greatest prey activity-densities occurring between May and August 2012 (Figure 2.8). Of the three target prey groups, availability of Collembola ($R^2 = 0.40$, $F_{1, 17} = 10.44$, $P = 0.005$) and Ensifera ($R^2 = 0.54$, $F_{1, 17} = 18.88$, $P = 0.0005$) were positively correlated with temperature, but availability of Diptera ($R^2 = 0.09$, $F_{1, 17} = 1.56$, $P = 0.23$) showed no association (Figure 2.7b).

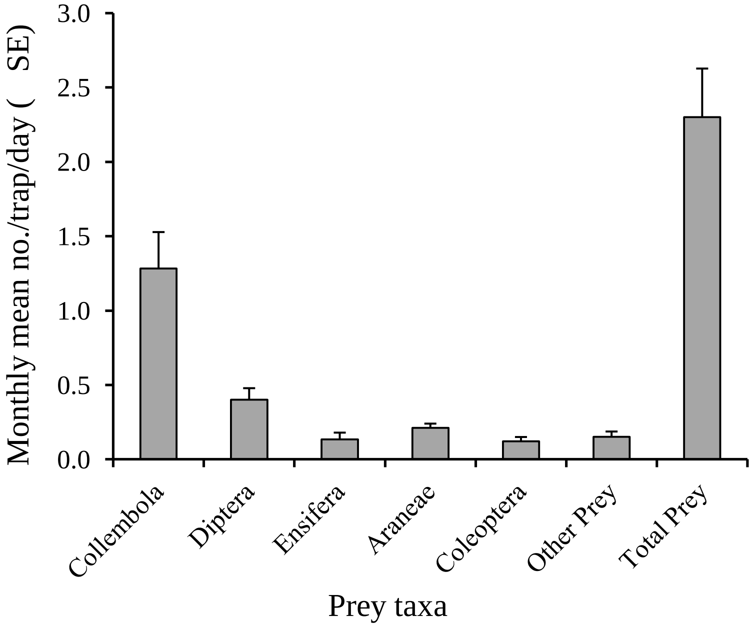

Figure 2.5 Mean (± SE) number of common potential prey captured (per trap/day) in pitfall traps between October 2011 and March 2013.

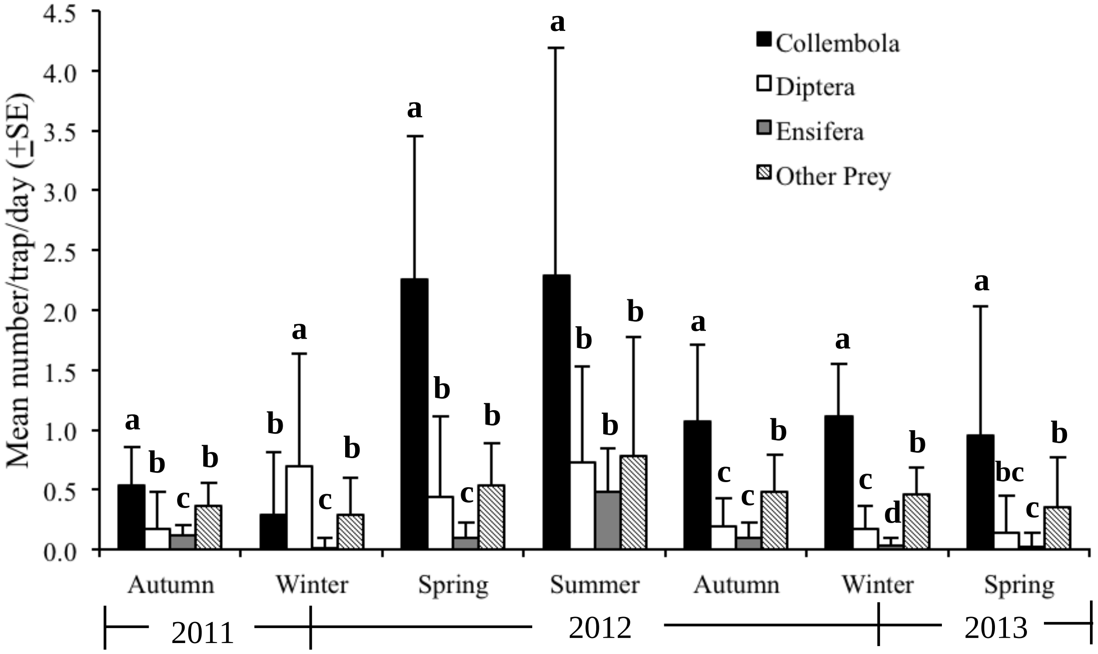

Figure 2.6 Mean (± SE) number of target and other potential prey captured (per trap/day) in pitfall traps separated by season. Separate one-way ANOVAs showed that availability among prey groups differed significantly within each season. Multiple comparisons were made using Tukey’s HSD, and significant differences within seasons are denoted by differing letters.

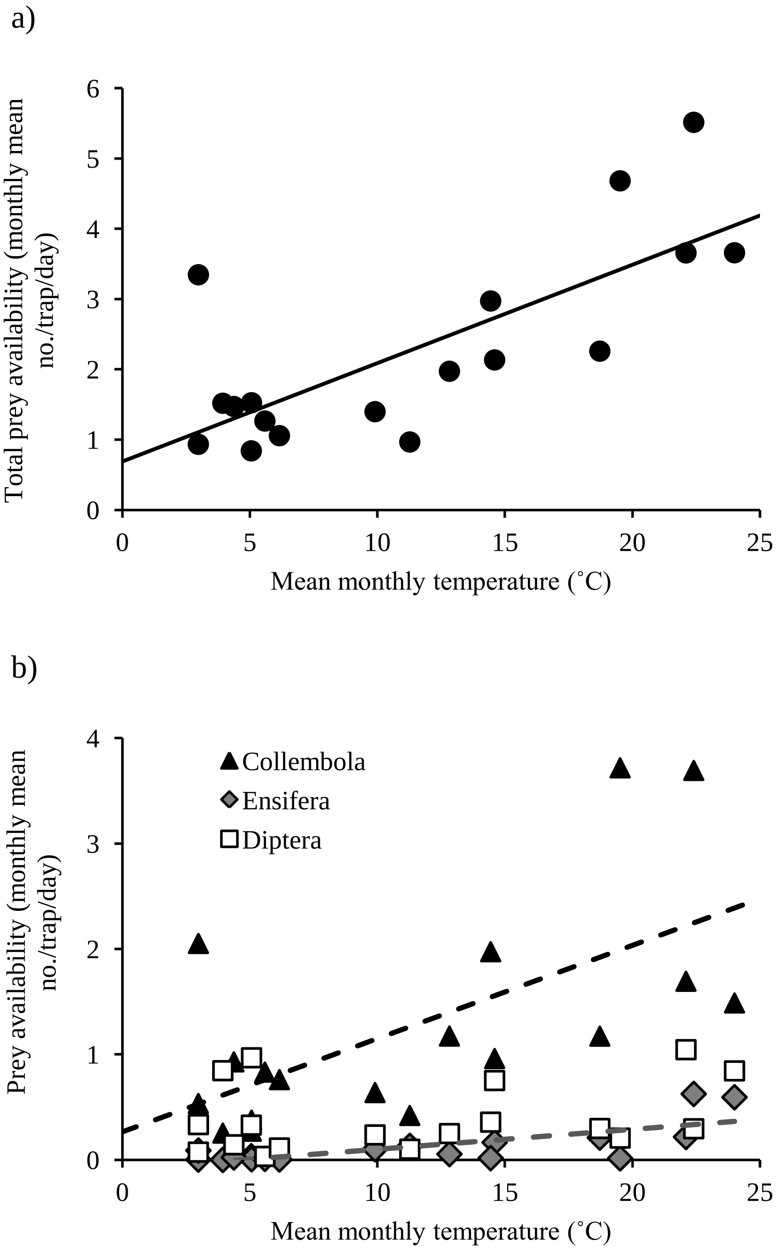

Figure 2.7 Linear regression of mean monthly temperature versus (a) total potential prey and (b) each target prey group. Regression lines denote a significant linear relationship.

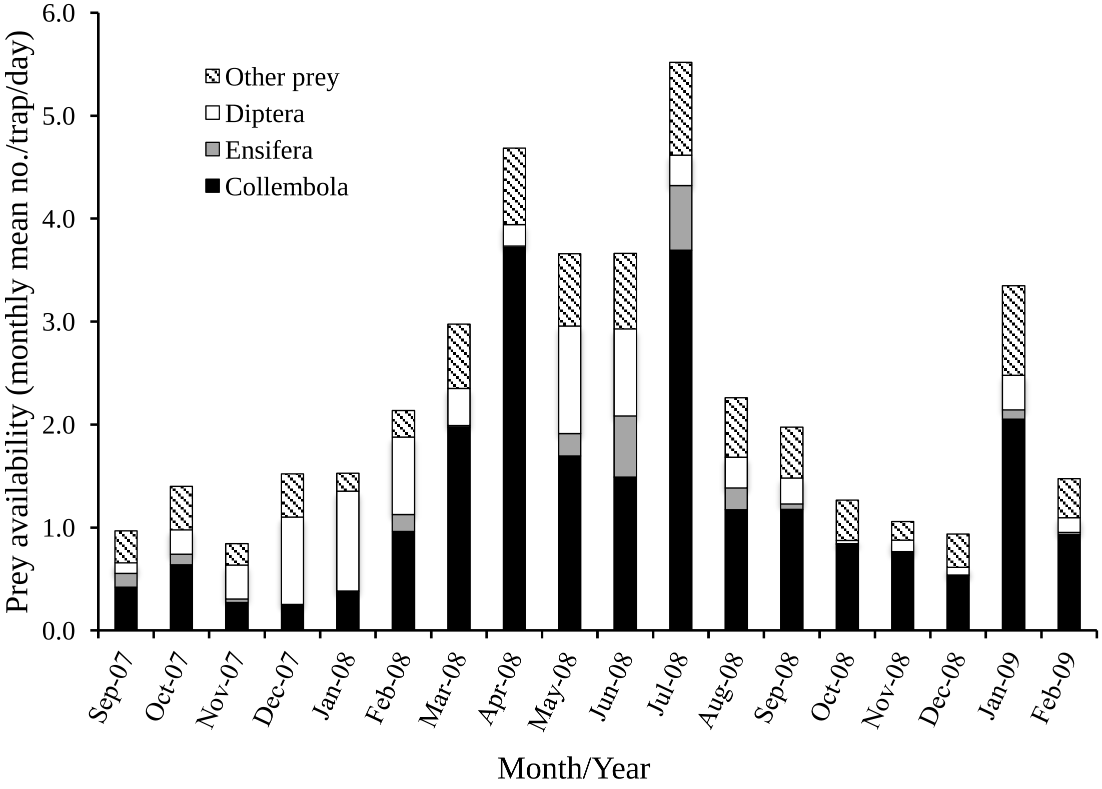

Figure 2.8 Mean (± SE) number of target and other potential prey captured (per trap/day) in pitfall traps separated by month.

2.4.3 Optimization of primers

The newly designed Ensifera primers, targeting *G. veletis*, *A. maculatus*, and *Ceuthophilus sp.*, amplified each taxa, respectively, and showed no cross-reactivity with each other, nor any other non-target taxa tested, including other Orthoptera (Appendix C). In addition, the primers generally specific to Collembola (Sint et al. 2012) and Diptera (Eitzinger et al. 2013) also only amplified DNA of springtails and flies, respectively, and did not amplify DNA from other non-target taxa.

2.4.4 Feeding trials and DNA detection

At 25 ˚C, the detectability of *D. melanogaster* within the guts of *S. ocreata* was 33% at 48 h. At 5 ˚C, detectability was 50% at 48 h. In both experiments, all control spiders, not fed *D. melanogaster*, did not test positive.

2.4.5 Molecular analysis of predation

A total of 1,231 spiders were collected between October 2011 and March 2013, 784 *S. ocreata* and 447 *S. stridulans*. Of all *Schizocosa* collected, 54 were male, 61 were female, and 1,116 were juvenile. Since the palps of immature spiders are not developed, sexing them at this stage is extremely difficult and is impossible in the field. Therefore, all juveniles were categorized together for analysis. All spiders were screened for the three most common prey taxa: Collembola, Diptera, and Ensifera.

Collembola were the most frequently consumed prey group throughout the study. About 44% of total spiders collected screened positive for Collembola DNA (n = 538), and between 15% and 71% of spiders screened positive in any given month (Figure 2.9a). In the logistic regression for this prey group, the model was significant ($\chi^2 = 60.23$, df = 5, $P < 0.0001$) (Table 2.2). The results showed that alternative prey availability, but not collembolan availability, significantly affected the probability of detecting Collembola DNA in the guts of *Schizocosa* (Table 2.2). Mean daily temperature was also negatively associated with Collembola predation (Table 2.2). Demographically, life stage had no effect, but species was associated: *S. stridulans* were more likely to consume Collembola than *S. ocreata* (Table 2.2).

Diptera were the second most frequently consumed prey group throughout the study. About 33% of total spiders collected screened positive for Diptera DNA (n = 402), and between 8% and 52% of spiders screened positive in any given month (Figure 2.9b). In the logistic regression for this prey group, the model was significant ($\chi^2 = 70.59$, df = 5, $P < 0.0001$) (Table 2.2). The results showed that neither dipteran availability nor alternative prey availability significantly affected the probability of detecting Diptera DNA in the guts of *Schizocosa* (Table 2.2). As in the analysis for Collembola, however, mean daily temperature was negatively associated with Diptera predation (Table 2.2). Similarly, life stage had no effect, but species was associated: *S. stridulans* were more likely to consume Diptera than *S. ocreata* (Table 2.2).

Of the three prey groups tested, ensiferans were the least frequently consumed. Only 3% of total spiders collected screened positive for Ensifera DNA (n = 40), and no more than 11% of spiders screened positive in any given month; in fact, there were seven separate months where no spider was detected with Ensifera DNA in their gut (Figure 2.9c). In the logistic regression for this prey group, the model was significant (χ^2 = 15.23, df = 5, P = 0.0094) and the fit was adequate (Pearson's χ^2 = 1148.43, df = 1223, P = 0.96) (Table 2.2). The results showed that there was no influence of Ensifera availability, alternative prey availability, nor mean temperature in predicting the probability of detecting ensiferan DNA in the guts of *Schizocosa* (Table 2.2). Again, *S. stridulans* were significantly more likely to consume this prey than *S. ocreata*, and in addition, adult spiders were marginally more prevalent Ensifera predators than juveniles (Table 2.2).

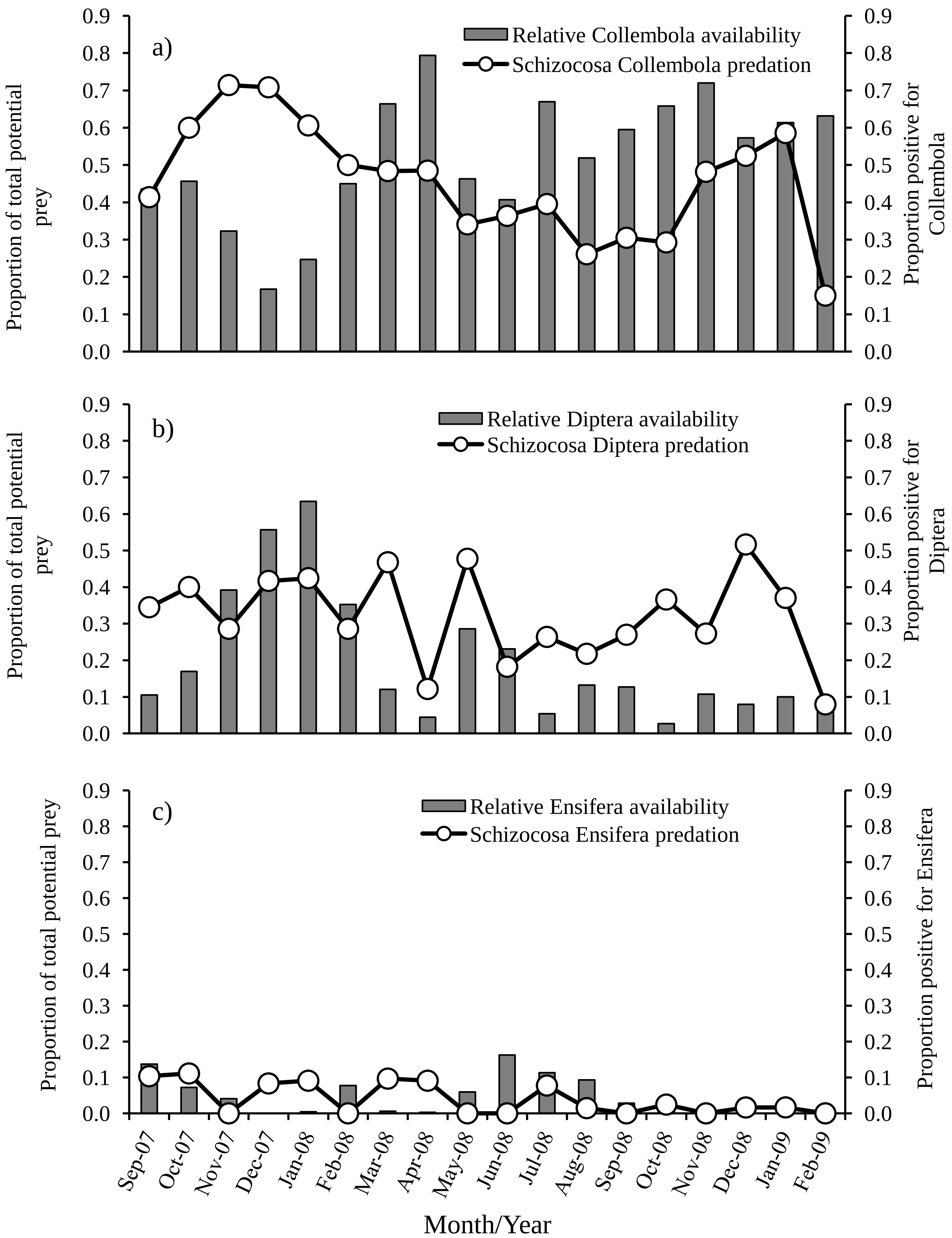

Figure 2.9 Comparison between temporal changes in prey availability and predation frequencies of (a) Collembola, (b) Diptera, and (c) Ensifera. Relative prey activity-densities of each prey (left axis, bars) were surveyed using pitfall traps and the proportion of *Schizocosa* spiders testing positive for DNA of each prey group (right axis, lines) was determined using PCR-based molecular gut-content analysis.

`Table 2.2 Results of multiple logistic regressions used to identify key factors that affected consumption by *Schizocosa ocreata* and *Schizocosa stridulans* on collembolans, dipterans, and ensiferans.

Prey Group	Parameter	Estimate ± SE	Likelihood-Ratio χ^2	P-value
Collembola	Intercept	0.30 ± 0.18	2.74	0.098
	Collembola availability	-0.09 ± 0.13	0.52	0.47
	Non-Coll. availability	0.45 ± 0.17	7.19	0.0073
	Mean temperature	-0.06 ± 0.01	43.77	<0.0001
	Species	-0.27 ± 0.06	18.70	<0.0001
	Stage	0.17 ± 0.11	2.26	0.13
Diptera	Intercept	0.049 ± 0.21	0.06	0.81
	Diptera availability	0.17 ± 0.25	0.44	0.51
	Non-Dipt. availability	0.002 ± 0.06	0.001	0.97
	Mean temperature	-0.05 ± 0.01	30.24	<0.0001
	Species	-0.38 ± 0.07	34.00	<0.0001
	Stage	0.20 ± 0.12	2.94	0.086
Ensifera	Intercept	-3.11 ± 0.61	34.14	<0.0001
	Ensifera availability	2.44 ± 1.45	2.90	0.089
	Non-Ens. availability	0.10 ± 0.18	0.32	0.57
	Mean temperature	-0.05 ± 0.03	2.08	0.15
	Species	-0.38 ± 0.17	4.99	0.0256
	Stage	0.48 ± 0.27	2.82	0.093

Similar to the observed seasonal trends in prey availability, there were also seasonal trends in spider foraging. Consistent with the result that temperature had a negative association with predation (Table 2.2), more spiders collected during winter months tested positive for Collembola ($\chi^2 = 37.05$, df = 1, $P < 0.0001$) or Diptera ($\chi^2 = 12.76$, df = 1, $P = 0.0003$) than those collected during months of other seasons (Figure 2.10). In addition, spiders that were found to have both Collembola and Diptera DNA within their guts (n = 226) were more likely to have been collected during winter months ($\chi^2 = 30.88$, df = 1, $P < 0.0001$) as well (Figure 2.10). Across the year, 42% of the spiders that fed on collembolans also consumed dipterans, and 56.2% of the spiders that fed on dipterans also consumed collembolans. Much more seldom occurring, spiders that screened positive for both Collembola and Ensifera (n = 20), Diptera and Ensifera (n = 16), and all three prey groups (n = 10), showed no significant difference in prevalence between seasons.

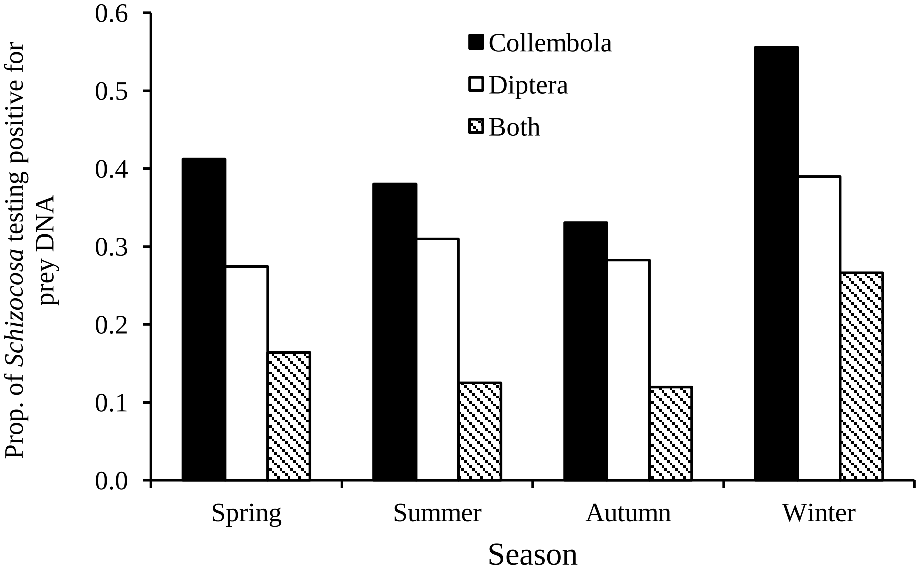

Figure 2.10 Proportion of *Schizocosa* spiders that consumed collembolans, dipterans, and both groups during different seasons. Spiders collected in the same month of different years were pooled into the same season.

Using the linear food selection index (Strauss 1979), it was possible to characterize spider selectivity for each prey group throughout the study (Figure 2.11). L_{Ensifera} values remained near zero in every month. The highest absolute L value of -0.162 was observed in July 2012, but during every other month, absolute L_{Ensifera} values were lower than ± 0.091. L_{Diptera} values were almost exclusively positive, but were marginally negative during March and July 2012 and more markedly negative during the three winter months of 2011-2012 (Figure 2.11). These negative values, as low as -0.21 in February 2012, were observed during the same months when relative dipteran availability was at its highest (Figure 2.9b). Compared with the second winter of 2012-2013, when relative availability of dipterans was much lower, dipteran predation frequencies changed little compared to the first winter (Figure 2.9b), which resulted in positive L_{Diptera} values, as high as 0.437 in January 2013 (Figure 2.11). In contrast to dipterans, $L_{\text{Collembola}}$ values were mostly negative to various degrees throughout the study, but were positive between November 2011 and March 2012 (Figure 2.11). Relative collembolan availability was at its lowest in winter 2011-2012, but collembolan predation frequencies were also at their highest (Figure 2.9a), which resulted in high $L_{\text{Collembola}}$ values of at least 0.359 during these months, as high as 0.541 in January 2012. By season, spiders tended to forage just as selectively, if not more so during winter months (absolute mean $L_{\text{Collembola}} = 0.267$, $L_{\text{Diptera}} = 0.222$) than during spring, summer and autumn months combined (absolute mean $L_{\text{Collembola}} = 0.212$, $L_{\text{Diptera}} = 0.167$).

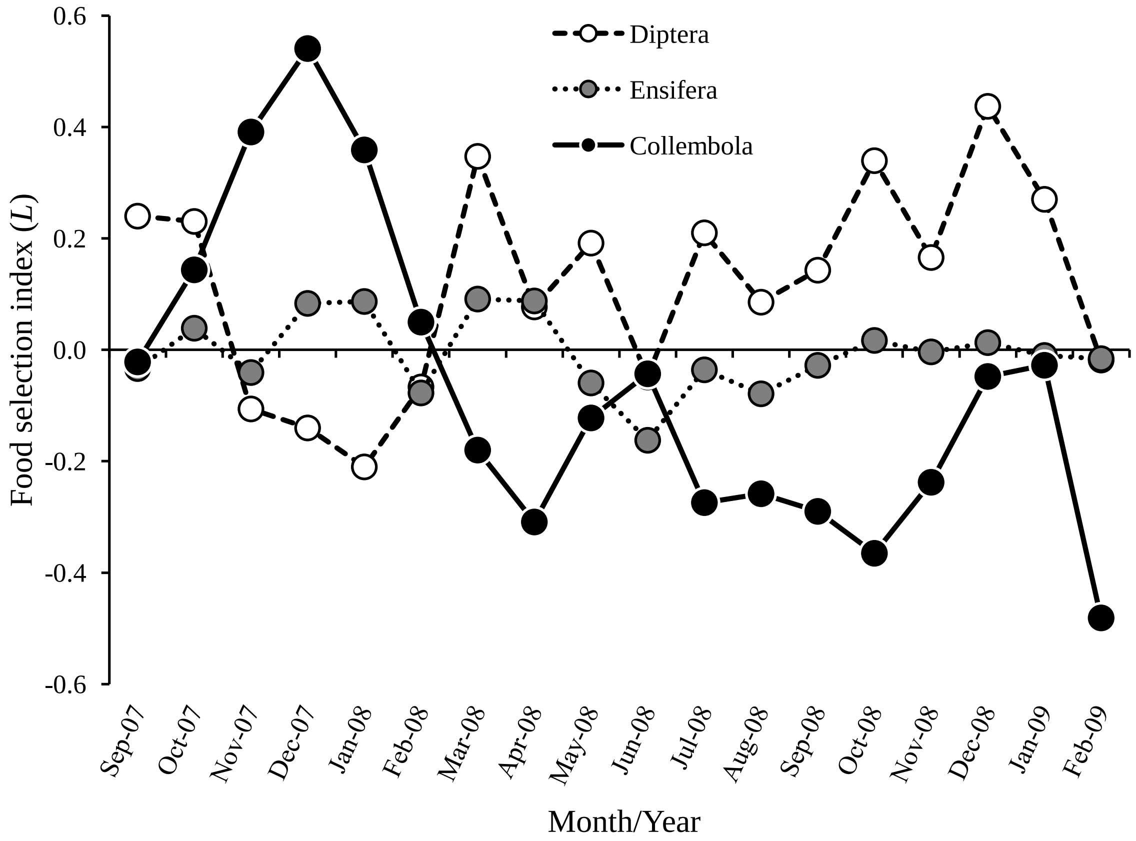

Figure 2.11 Month-by-month tracking of selective feeding behavior in *Schizocosa* spiders using the linear food selection index, $L = r_i - p_i$ (Strauss 1979), where r_i was the proportion of spiders feeding on prey *i*, and p_i was the proportion that prey *i* occurred in pitfall traps compared to other potential prey. Positive *L* values indicate preference, negative *L* values indicate non-preference or avoidance, and an *L* value of zero indicates random, frequency dependent predation of prey *i*.

2.5 Discussion

Contrary to my hypothesis based on optimal foraging theory, there was little evidence to support the notion that prey availability governs foraging behavior in these generalist predators. In all three cases, the activity-densities of Collembola, Diptera, and Ensifera had no significant influence on predicting the probability of *S. ocreata* or *S. stridulans* screening positive for DNA of each target prey group, respectively (Table 2.2). In fact, non-collembolan prey availability was observed to have a significant negative association with Collembola predation, meaning that when *Schizocosa* were exposed to more prey possibilities, they fed more heavily on collembolans. This is not characteristic of a strictly opportunistic forager, which primarily seeks to maximize prey capture rate. Rather, this suggests that these spiders are capable of making selective feeding decisions in the field, consuming prey independent of its availability, which has also been displayed in other lycosids (Kuusk and Ekbom 2012). The ways by which they make foraging decisions, however, requires further investigation, especially because *Schizocosa* rely mainly on vibrations to sense and identify appropriate prey (Foelix 2011). Although their eyesight is better than spiders of several other families, it is most important for judging mating displays and is not heavily relied on in foraging to identify prey to select or avoid (Foelix 2011). Instead, it is possible that these spiders select prey by killing first and choosing later. Unlike chewing invertebrates, spiders have been shown to be capable of selective nutrient extraction within a single individual prey item (Mayntz et al. 2005, Wilder 2011), and, in addition, lycosids are also known to only partially consume individual prey under certain circumstances, referred to as “wasteful killing” (Samu and Biro 1993). Therefore, it stands to reason that *Schizocosa* do not premeditate which prey to pursue, but may express selective choices for prey primarily after indiscriminate capture events, depending on nutritional composition.

On a temporal scale, *Schizocosa* did not appear to forage any more selectively during the spring, summer, and autumn than during the winter (Figure 2.11), despite the overall availability of prey significantly decreasing with temperature (Figure 2.7a). I predicted that a more limited prey resource environment during the winters would foster a higher degree of frequency-dependent feeding in spiders, and conversely a richer prey resource environment between the spring and autumn would allow spiders to be more deliberate about what they consume. What I observed, however, was that *Schizocosa* exhibited higher absolute $L_{\text{Collembola}}$ and L_{Diptera} values during winter months compared to during warmer months when invertebrate activity-densities were much higher (Figure 2.11). This suggested that these spiders were not seasonally limited in their ability to selectively forage, as was hypothesized. Moreover, this provided further evidence that availability of prey did not strictly dictate the foraging tendencies in these generalist predators.

Selective and frequency-dependent feeding behaviors were not mutually exclusive, however. *S. ocreata* and *S. stridulans* exhibited both foraging responses to prey availability depending on the prey type. In the case of Ensifera, for example, spiders seldom encountered and also seldom consumed crickets throughout the study (Figure 2.9c), which consistently yielded L_{Ensifera} values close to zero (Figure 2.11). Thus, unlike collembolans and dipterans, spiders fed on ensiferans in a frequency-dependent manner, appearing neither to completely avoid, nor completely focus on this more uncommon prey group. Given these results, the three Ensifera species that were tested were presumably neither economically profitable enough to be extensively sought after, nor economically detrimental enough to be completely ignored. Nevertheless, these spiders did include these more intermittently accessible prey species to their diet when given the opportunity.

In general, diet diversification increases most spider fitness parameters in laboratory experiments (Uetz 1992, Toft 1995, Toft and Wise 1999, Harwood et al. 2009), and the results from this field study demonstrate that *S. ocreata* and *S. stridulans* put this optimal foraging strategy into practice in nature. Out of the 728 spiders observed with target prey DNA in their guts, 272 (37%) consumed multiple prey types. Unsurprisingly, Collembola were the most important spider prey of these three groups; not only were they consumed at an overall higher rate throughout the study (Figure 2.9a), I observed that 56% of the spiders that consumed dipterans and 90% of the spiders that consumed ensiferans, had also consumed collembolans. Collembola have been shown to be vital prey animals for several generalist arthropod predators, including cantharid beetles (Eitzinger and Traugott 2011), carabid beetles (Pollet and Desender 1987, Bilde et al. 2000, Eitzinger and Traugott 2011), linyphiid spiders (Marcussen et al. 1999, Harwood et al. 2004, Romero and Harwood 2010), and also lycosid spiders, specifically *Schizocosa ocreata* (Toft and Wise 1999). Large bodied species that are commonly found in forest leaf litter, especially from the families Tomoceridae and Entomobryidae, are exceptionally high in nutritional quality for spiders (Toft and Wise 1999, Rickers et al. 2006). Diptera are lesser known to be a major component in lycosid diets, mainly because their alate physiology is not thought to be conducive to epigeal predator capture. Kruse et al. (2008), however, showed that the flexible bodies, legs, and chelicerae of lycosids are highly adapted to catching dipterans, and additionally, other studies have observed lycosids to consume adult dipterans in equal or greater proportions to collembolans in some systems (e.g. Bardwell and Averill 1997, Morse 1997, Ishijima et al. 2006). Although most Diptera are lower in nutritional value, they are still beneficial for these spiders to consume, because they are rich in protein (Marden 1989, McLachlan and Neems 1996, Mayntz and Toft 2001). Toft and Wise (1999) found dipterans to be particularly beneficial for *S. ocreata* when supplemented with collembolans, because the combination promoted more growth than any single-species diet. Furthermore, *S. ocreata* and *S. stridulans* appeared to selectively forage for collembolans and dipterans in a way that balanced their intake, according to the monthly linear food selection index values (Figure 2.11). For instance, during the first winter months of 2011-2012, $L_{\text{Collembola}}$ values were positive and L_{Diptera} values were negative. During this time, relative Collembola activity-densities were at their lowest, but Collembola predation frequencies were at their highest (Figure 2.9a). This strong preference toward Collembola is likely occurring to meet basic requirements during this brief exposure to a suboptimal resource base, saturated with lower quality prey. When Collembola were higher in availability during the second winter of 2012-2013, however, spiders likely were more easily able to obtain this integral prey resource, which would explain why $L_{\text{Collembola}}$ values were closer to zero during this time. Moreover, despite relative Diptera activity-densities being at their highest values during the first winter months, spiders did not markedly increase their fly predation compared to adjacent seasons (Figure 2.9b). Throughout most of the remainder of the study, however, activity-densities of Collembola were much higher than Diptera (Figure 2.6), and as a result, L_{Diptera} values were greater than $L_{\text{Collembola}}$ values. These results indicate that, in general, when one of these two main dietary resources is in higher abundance, *Schizocosa* tend to consume it at a less than random rate in order to focus more foraging attention toward the other that is in lower abundance. This clearly displays prey switching behavior, which is common among generalist predators (Murdoch 1969), but more interestingly, this provides compelling evidence that the strategy of diet diversification, found to be advantageous for *S. ocreata* in artificial laboratory environments (Toft and Wise 1999), is occurring in the field.

During the winter, both *S. ocreata* and *S. stridulans* are active as juveniles, consuming prey during mild periods (Aitchison 1984a) for steady growth to expedite spring maturation. Incorporating a mixture of prey types into their diet may be especially important for these spiders to maximize their growth potential and minimize their maturity time before spring. This is supported by the fact that (1) spiders tested positive for Collembola and Diptera DNA at a higher frequency during winter over any other season (Figure 2.11), and (2) temperature had a significant negative association in predicting spider predation of these two groups (Table 2.2). Besides growth, the findings that spiders exhibit higher rates of predation in cooler weather have a few other possible explanations as well. From a biological perspective, the effectiveness of Collembola furca to escape capture may be compromised at low temperatures (Boiteau and MacKinley 2012), which would allow spiders to more easily subdue these prey and would help corroborate the higher collembolan predation rates detected during winter. Additionally, larger *Schizocosa*, active during the spring and summer, may take larger prey, perhaps of taxa not tested in this study, at a higher rate than smaller spiders during the winter. Small and easily subdued collembolans and dipterans may be the extent of the prey groups these immature spiders were willing to pursue during harsh winter conditions. In addition to these possible biological explanations, methodology may also help to explain the effect of temperature on predation. This is mainly due to the fact that varying ambient temperatures can alter the retention time of DNA within the guts of predators (Hoogendoorn and Heimpel 2001, von Berg et al. 2008), including lycosid spiders (Kobayashi et al. 2011). Given that arthropods are ectothermic, low temperatures force their metabolism to decelerate, which can result in slower digestion. This can be a potential issue when interpreting molecularly derived food web data on a seasonal basis. The reduced metabolic rate of spiders collected during winter months may have slowed the decay of prey DNA within their alimentary canals, perhaps causing slightly more positive readings to be observed during PCR. The DNA detection feeding trials showed this to be true after 48 h, but not to an overwhelming degree. Given this and the strength of the observed trends in the field, it is likely that any possible differences in DNA retention across seasons is negligible, solidifying the fact that temperature was a strong predictor of Collembola and Diptera predation in *Schizocosa*. In spite of the potential limitations of PCR-based molecular gut-content analysis, this technique succeeded in further elucidating the foraging dynamics of these generalist predators during an understudied portion of the year.

In conclusion, this research provided evidence that *S. ocreata* and *S. stridulans* are capable of making selective feeding decisions in their leaf litter habitat, which they exhibit across a spectrum of seasonal change in prey availability. This contradicts previous notions stemming from OFT that generalist predators are indiscriminant foragers, especially when prey is scarce. Additionally, *Schizocosa* applied these selective feeding decisions in a way that seemed to diversify their diets, which has previously been shown to improve their fitness. This study was the first to track prey choice in spiders according to seasonal prey availability, but more research would be helpful to further elucidate *Schizocosa* trophic dynamics. For example, to add further insight into the temporal nuances of prey choice, more frequent sampling of prey activity-densities and more frequent collection of predators for gut-content analysis would be ideal for future studies. Although this research focused on the temporal aspect of prey availability and predator foraging, varying prey population density in space is also likely to influence *Schizocosa* foraging decisions. Especially in sit-and-wait spiders, patch dynamics are extremely important (Wise 1993). Spiders generally strive to forage in prey-rich patches (Harwood et al. 2001, 2003, Welch et al. 2013), and must also decide how long to exploit a patch (Gillespie and Caraco 1987). Thus, using a distinct spatial organization to sample both prey availability and predation frequencies may reveal new information about how prey aggregations affect spiders. Lastly, in addition to strengthening the spatio-temporal components, expanding on the number of prey groups to use in molecular gut-content analysis would add resolution to the food web. The implications for trophic dynamics would be particularly interesting if future studies investigated intraguild predation. These *Schizocosa* species often exhibit cannibalism (Wise and Wagner 1992, 1997), and other spiders were observed in high abundance throughout the year in the field site (Figure 2.5), so there seems to be potential for trophic omnivory to occur.

Chapter 3: Tradeoff in winter-active wolf spiders: increased mortality for increased growth

3.1 Summary

A number of arthropods, including some spiders, forgo diapause as an overwintering strategy and are instead active at low temperatures. The higher metabolic rates and periodic foraging behavior associated with winter activity, however, can be incompatible with high levels of freeze avoidance. This is thought to be due, in part, to increased ice nucleators in the gut after feeding events and physiological complications brought on by accumulating certain cryoprotectants. To characterize the relationship between winter activity and cold hardiness, this study quantified the seasonal resistance to freezing in two common wolf spiders of hardwood forests in the Eastern United States, *Schizocosa ocreata* and *Schizocosa stridulans* (Araneae: Lycosidae). Individual spiders were collected from a deciduous forest in Kentucky from August 2012 to March 2013 and were subjected to supercooling point (SCP) determination assays. Contrary to many invertebrates with high cold hardiness, mean SCP of *S. ocreata* and *S. stridulans* remained constant throughout the study, which was subsequently determined to be their lower lethal temperature. Interestingly, daily low temperatures within the leaf litter occasionally fell below the mean SCP of the spiders, subjecting them to a significant risk of freezing during winter. To determine if this high risk was a result of winter predation, spiders were fed varying quantities of prey, but no significant association between consumption and SCP was found. Despite exposure to potentially lethal temperatures, *Schizocosa* did not seasonally augment their cold hardiness to better survive. This suggests an ecological tradeoff, where these spiders appear to assume increased mortality risk in exchange for maximized growth opportunity during a time of year when few competitors are active. It was reasoned that the increased fitness benefits associated with early maturation and larger size in spring help to sustain these abundant spider populations.

3.2 Introduction

Temperate environments are typically characterized by wide seasonal variation in temperature and are home to a myriad of ectothermic animals that employ a host of adaptations to survive winter. For example, some members of the Arthropoda have adapted tolerance to internal freezing to overcome exposure to sub-zero temperatures (Doucet et al. 2009), but most species can be considered as “freeze-avoiding” (Sinclair et al. 2003). These arthropods must remain unfrozen at low temperatures, which requires them to maintain their hemolymph in a liquid state below its freezing point, a process termed supercooling (Salt 1961). The temperature to which an arthropod cannot continue supercooling, when ice crystallization of its body fluids occurs, is called the supercooling point (SCP) and is often used as a metric for cold hardiness. There are several physiological mechanisms to decrease SCP and increase survivorship in overwintering arthropods, one of which involves limiting the concentration of ice nucleating agents in the body. Commonly manifested as proteins, bacteria and/or dust particles, ice nucleators act to accelerate spontaneous ice crystallization and thus increase SCP when in high concentrations (Duman 2001, Bale 2002, Lundheim 2002). Although ice nucleators can be produced internally, a common external source is from ingested food (Block and Zettel 1980). Therefore, in preparation for winter many arthropods will either void their guts and/or enter a period of starvation to maintain a low SCP (Danks 1978, Somme 1982, Duman et al. 1991). Furthermore, the accumulation of cryoprotectants within the body is another common adaptation for arthropods to survive the winter. Frequently initiated in response to shorter photoperiods and decreasing temperatures, many will produce and incorporate ice nucleator-inhibiting or osmotic pressure-elevating chemicals to prevent ice formation (Duman 1979, Lee 2010).

Adaptations for surviving cold weather are well documented in insects (Storey and Storey 2012) and, to a lesser extent, spiders (Somme 1982). In the limited number of spiders studied to date, all are documented as freeze-intolerant (Schaefer 1977) and many are susceptible to mortality without freezing after prolonged cooling periods (Kirchner and Kestler 1969, Schaefer 1976, Danks 1978, Lee et al. 1987). Consequently, spiders must supercool their body fluids to avoid the fatal formation of ice crystals. However, a distinction between the level of freeze resistance and the level of adaptive response to low temperatures is evident within Araneae according to Kirchner (1987). In this review, it was observed that only spiders with high cold hardiness (i.e. SCP values -16˚C to -34˚C) experienced seasonal decreases in SCP during winter, whereas spiders with low or moderate cold hardiness (i.e. SCP values -4 to -16˚C) tended to have constant SCPs throughout the winter. Since SCP depression is the result of an adaptive physiological change, this implies that spiders with low or moderate cold hardiness do not accumulate cryoprotectants. To compensate for their susceptibility to low temperatures, these spiders rely on behavioral adaptations, such as finding insulating hibernacula (Schaefer 1977, Kirchner 1987), which can be favorable given the high cost of cryoprotectant production (Duman et al. 1991).

Spiders primarily overwinter in a state of diapause, but some species are winter-active and exhibit only temporary quiescence when temperatures fall below a given threshold (Schaefer 1977). These spiders characteristically exhibit opportunistic foraging during mild periods of winter (Huhta and Viramo 1979, Aitchison 1987, Korenko et al. 2010), which is not only necessary to meet the energy requirements of a higher metabolism, but also provides an opportunity for growth (Aitchison 1984a). Despite reduced prey availability during winter, when predator diets typically consist of winter-active collembolans and dipterans (Aitchison 1984a, Nentwig 1987, Eitzinger and Traugott 2011, Jaskula and Soszynska-Maj 2011), fewer competitors for these resources are present (Schaefer 1977, Kirchner 1987). Intraguild predation is still a mortality factor for some winter-active spiders (Gunnarsson 1985, Korenko and Pekar 2010), but generally, if foraging is successful and winter growth is achieved, there may be a competitive advantage to occupying this niche, because high levels of fitness favor spiders that mature quickly and are large (Gunnarsson 1988). Conversely, prey consumption can compromise the cold hardiness in arthropods through increasing the number of ice nucleators in the gut (see examples in Somme 1982), and also, it has been suggested that the incorporation of certain cryoprotectants causes physiological constraints on arthropods by hindering locomotive and active foraging ability (Vanin et al. 2008). Therefore, to minimize winter mortality, which is a primary factor influencing spider population dynamics (Schaefer 1977), there exists a possibility that winter-active species must balance the benefits of foraging with the increased risks of freeze-susceptibility.

In this study, I examined the relationship between winter-feeding and seasonal freeze resistance by tracking changes in the cold hardiness of two winter-active wolf spiders, *Schizocosa ocreata* (Hentz) and *Schizocosa stridulans* (Stratton) (Araneae: Lycosidae). These spiders are abundant leaf litter predators and are widespread across eastern deciduous forests of the Nearctic (Stratton 1991). Although members of the genus have been extensively studied for their courtship behaviors (e.g. Uetz and Roberts 2002, Clark et al. 2011, Uetz et al. 2013) and to some extent their predation dynamics (e.g. Toft and Wise 1999, Wise and Chen 1999) and impact on ecosystem processes (e.g. Lensing and Wise 2006), there is a paucity of information pertaining to their cold hardiness and winter-feeding ecology. Both *S. ocreata* and *S. stridulans* are known to overwinter as juveniles and exhibit regular feeding during this time (Figure 2.9), but it is merely speculation as to how foraging behavior is associated with their low temperature adaptations. The goal of this research was to determine the physiological capacity of *Schizocosa* to resist freezing and identify any effects of prey consumption on their cold hardiness. Based on the pattern reported by Kirchner (1987) and given the relative mildness of winters these spiders endure across their range, I hypothesized that *S. ocreata* and *S. stridulans* would not adaptively suppress their SCPs in preparation for winter. In addition, I predicted that if winter-feeding increases the concentration of ice nucleators in the spider gut, SCP would increase after feeding events and negatively impact cold hardiness.

3.3 Materials and Methods

3.3.1 Seasonal monitoring of spider supercooling points

All spiders were collected from Berea College Forest in Madison County, Kentucky, USA (37˚34’22”N, 64˚13’11”W, elevation ~ 268 m), where air temperatures range from mean winter lows of -2.8 ˚C to mean summer highs of 29.4 ˚C (National

Weather Service; www.weather.gov). The leaf litter is structurally complex and provides excellent hibernacula for invertebrates from extreme temperatures (Edgar and Loenen 1974, Kraus and Morse 2005). Given the likely variation in litter temperature compared to air temperature, HOBO® Pro v2 data loggers (Onset, Bourne, Massachusetts, USA), as in the previous chapter of this thesis, were positioned 1 cm above the soil surface to monitor temperatures experienced by spiders in their epigeal habitat (Figure 2.2). Temperature measurements were recorded hourly during the study period, and daily low temperatures were used to compare and contextualize observed SCPs of field-collected spiders.

Schizocosa ocreata and *S. stridulans* were hand-collected monthly from August 2012 to March 2013 to track seasonal changes in SCP, allowing for a comparison of their cold hardiness before, during, and after the winter season. As in the previous chapter, collections occurred at night using headlamps; the eyes of *Schizocosa* reflect light, which facilitated easy location of individuals. Spiders were removed from the litter using an aspirator, placed in separate 2.0 mL microcentrifuge tubes and returned to the laboratory to determine mass and supercooling point of each individual (described in detail below).

3.3.2 Supercooling point determination

Individual spiders were transferred into 2.0 mL microcentrifuge tubes containing a copper-constantan thermocouple connected to USB Thermocouple Data Acquisition Module (TC-08; OMEGA Engineering, Stamford, Connecticut, USA). Dry cotton was added to each tube to ensure the spiders were in contact with the tip of the thermocouple (Figure 3.1a). Samples were placed into 50 mL conical tubes suspended in an ethylene glycol cold bath (NESLAB RTE-740, Thermo Scientific, Waltham, Massachusetts, USA) set to 5 °C (Figure 3.1b). Once all the samples standardized to approximately 5 °C, the bath was cooled from 5 °C to -25 °C at a rate of 0.5 °C min^{-1}. The supercooling point for each individual was determined from the latent heat of crystallization (after Lee 1989) identified from temperature measurements recorded by OMEGA data acquisition software (OMEGA Engineering). Mean SCPs of each species were transformed by rank to meet the assumption of normality, and the effects of collection date, spider species, and their interaction were determined using a two-way ANOVA. Multiple comparisons were made using Tukey's HSD.

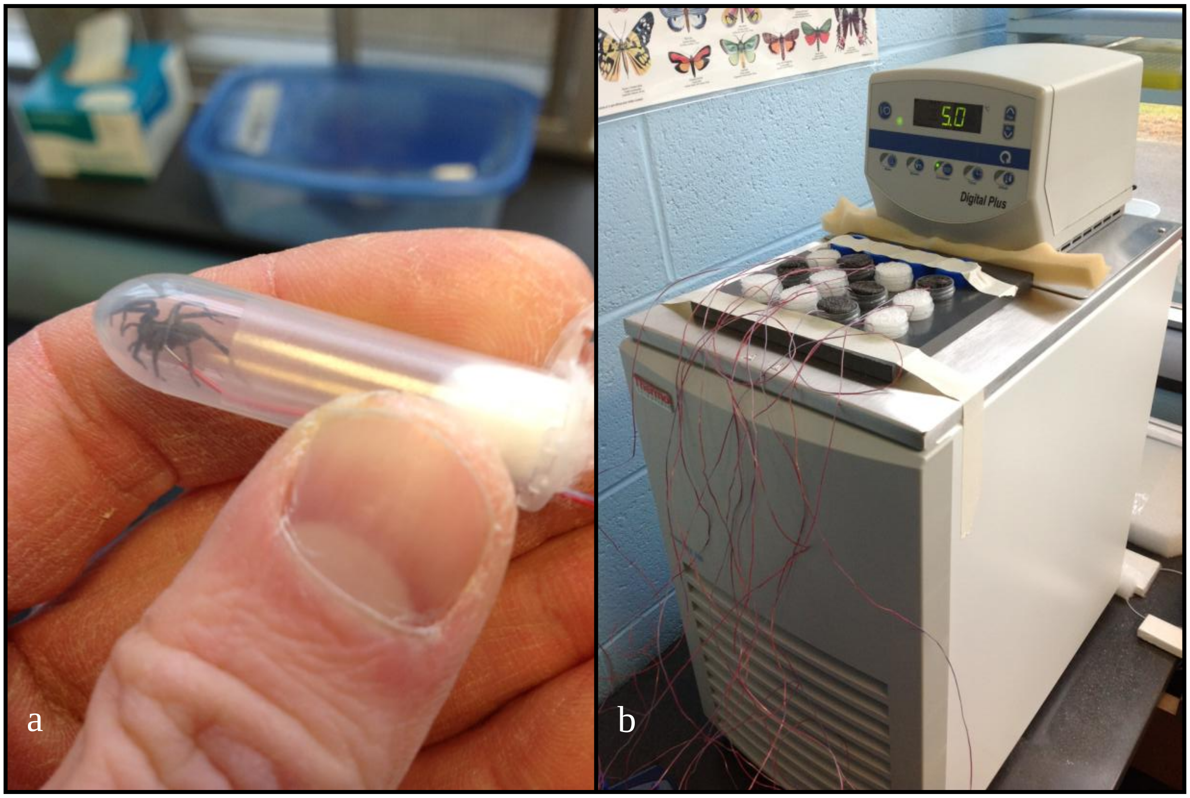

Figure 3.1 Supercooling point (SCP) determination assay. Spiders were connected to thermocouples (a) and subjected to a cooling bath (b), where their internal temperatures were monitored for the release of latent heat that signifies the phase transition between liquid to solid.

3.3.3 Low temperature survival assay

Juvenile *S. stridulans* were collected from the field to compare the survival of spiders after freezing or supercooling at -7 ˚C, a temperature near the mean SCP of the spiders. As before, spiders were placed into 2.0 mL microcentrifuge tubes with thermocouples. However, in contrast to the methods described above, either wet or dry cotton (total n = 40) was added to each tube; dry cotton was included to permit spiders to supercool, whereas wet cotton was added to serve as a site of external ice nucleation to induce freezing (after Kostal et al. 2012). All samples were placed into conical tubes and suspended in an ethylene glycol cold bath set to -7 ˚C for 90 min. As individuals were attached to thermocouples, only those not displaying an exotherm were included in the supercooled group, whereas those exhibiting an exotherm were included in the frozen group. After 90 min, spiders were removed from the cold bath and subsequent movement was assessed following a 30 min recovery at room temperature.

3.3.4 Effect of prey consumption amount on supercooling point

Juvenile *S. ocreata* were collected from the field and placed in separate containers with a moistened base layer of plaster of Paris. Individuals were maintained at 6 ˚C on a 11L:13D cycle, comparable to the natural conditions of a mild winter day when most spiders were collected and foraging is more common (evidence suggests that despite being winter-active, these spiders feed infrequently at near-zero degree temperatures (Aitchison 1984, Korenko et al. 2010), justifying the temperature selected). Spiders were separated into four experimental groups and either starved or fed one, two or three *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) over three days (n ~ 10 per treatment). The mass of each spider was measured before and after the feeding period to compare weight gain to cold hardiness. Spiders were then subjected to SCP determination, as outlined above, and mean SCPs between treatments were compared using one-way ANOVA.

3.4 Results

3.4.1 Seasonal monitoring of spider supercooling point and mass

The mean SCP in individual spiders did not differ between species tested ($F_{1, 172}$ = 0.01, P = 0.99). *S. ocreata* and *S. stridulans* supercooled to an average temperature of -7.57 ± 0.92 ˚C (n = 135) and -7.75 ± 0.98 ˚C (n = 49) respectively, before internal ice formation occurred. By collection date, mean SCP differed significantly ($F_{7, 172}$ = 6.43, P = 0.0001) (Figure 3.2), but interestingly it did not decrease during the winter. Rather, spiders had lower SCPs in August and September compared to November, January and March (Tukey's HSD to compare means between collection dates). Regardless of these subtle, but significant changes, SCP remained relatively stable throughout the monitoring period with no significant differences observed between other dates. The interaction effect between collection date and species was also significant ($F_{7,\ 172} = 2.16$, $P = 0.04$).

Daily low temperatures within the leaf litter ranged from 23.5 ˚C in late summer to -10.4 ˚C in mid-winter (Figure 3.3). On several days during the study period, temperatures fell within a few degrees of the overall mean SCP of both *Schizocosa* species (-7.62 ± 0.97 ˚C), and on four days fell below this threshold; in one instance temperature in the leaf litter was 2.8 ˚C below spider SCP. Variation of SCP within the population was positively skewed (Figure 3.4), which showed that while the majority of spiders experienced freezing risk, some individuals were better adapted for these temperatures. Mean body mass increased significantly from August to March in nymphal *S. ocreata* ($R^2 = 0.72$, $F_{1,\ 6} = 13.11$, $P = 0.015$) and *S. stridulans* ($R^2 = 0.77$, $F_{1,\ 6} = 17.03$, $P = 0.009$) (Figure 3.5)

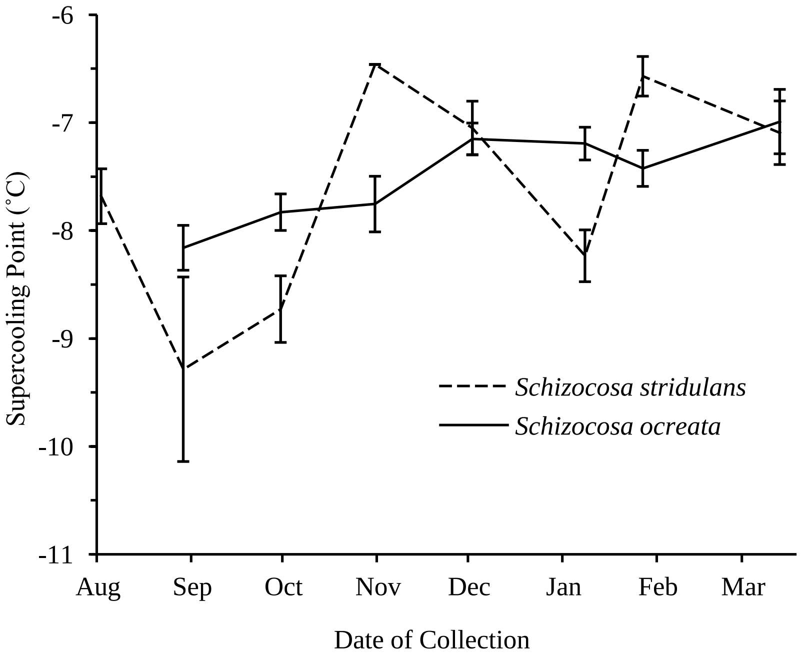

Figure 3.2 Mean (± SE) supercooling points of *Schizocosa stridulans* and *Schizocosa ocreata* from late summer 2012 to early spring 2013.

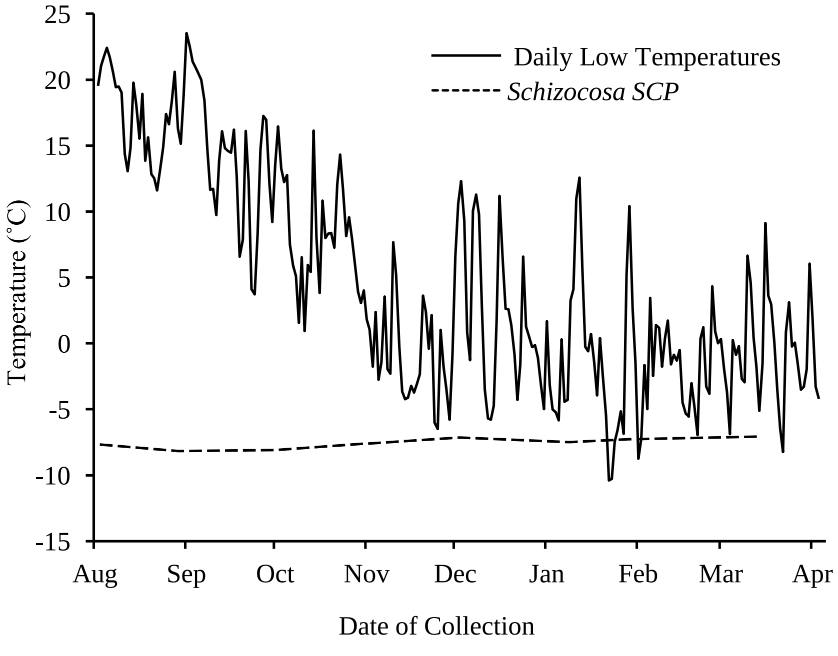

Figure 3.3 Daily minimum leaf litter temperature and mean *Schizocosa* supercooling point from late summer 2012 to early spring 2013.

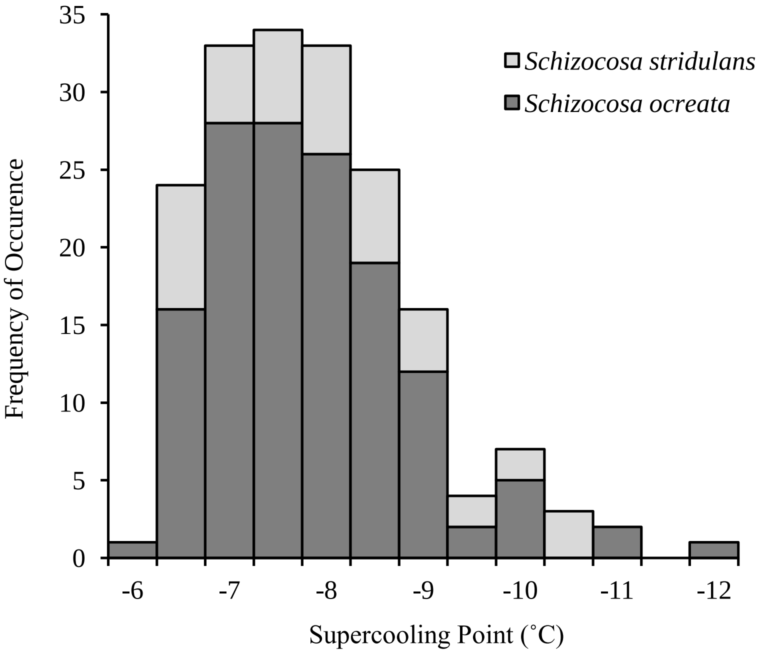

Figure 3.4 Frequency distribution of supercooling points in *Schizocosa ocreata* and *Schizocosa stridulans*.

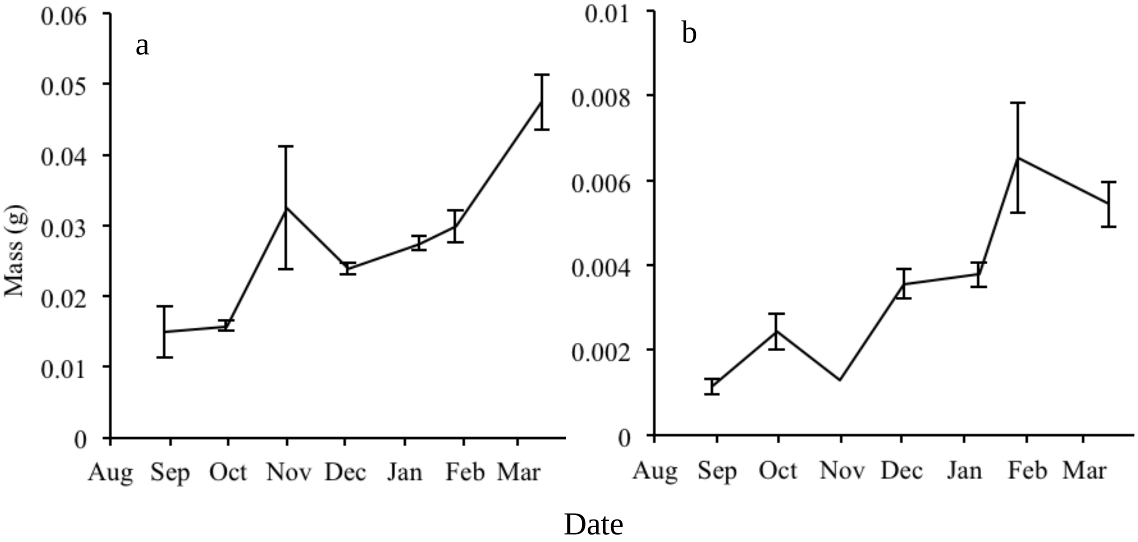

Figure 3.5 Increasing mass occurring between late summer and early spring in juvenile (a) *Schizocosa ocreata* and (b) *Schizocosa stridulans*.

3.4.2 Low temperature survival assay

Survival of *S. stridulans* differed significantly between individuals subjected to the dry cotton treatment (which permitted spiders to supercool) versus the wet cotton treatment (which served as a site of ice nucleation) (χ^2 = 13.5, P = 0.0002). Spiders that froze experienced 100% mortality compared to 53% of the non-frozen individuals.

3.4.3 Effect of prey consumption on supercooling point

Spider weight gain was correlated to consumption of Drosophila (one-tailed t = 2.43, P = 0.012) but not to SCP (R^2 = 0.008, F = 0.31, P = 0.58). Moreover, the quantity of prey consumed did not affect the SCP of *S. ocreata* ($F_{3, 41}$ = 1.43, P = 0.25) (Figure 3.6).

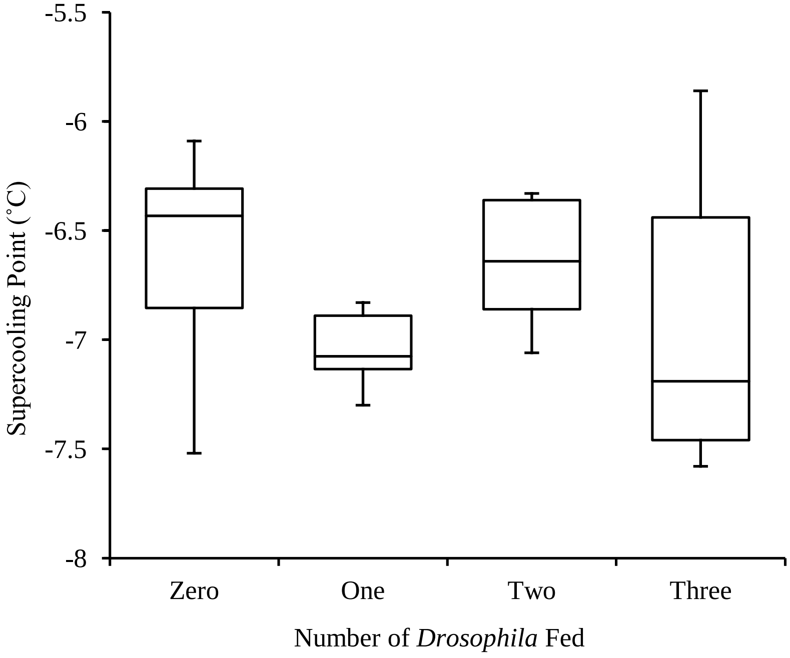

Figure 3.6 Box-and-whiskers plot of the supercoiling point of *Schizocosa ocreata* fed zero, one, two and three *Drosophila melanogaster*.

3.5 Discussion

Unlike many winter-active arthropods (e.g. Watanabe 2002, Crosthwaite et al. 2011, Khodayari et al. 2013), *S. ocreata* and *S. stridulans* displayed no adaptive SCP changes in response to seasonal temperature shifts (Figure 3.2). Instead, mean SCP for both species remained relatively stable from summer to spring and was high in overall value relative to SCPs of spiders inhabiting more northern latitudes (Kirchner 1973, Schaefer 1976). These findings are consistent with the pattern found in Kirchner (1987), where winter SCP depression was found to occur in spiders with high cold hardiness, but not in those with low cold hardiness. Both species of *Schizocosa* can be considered examples of those spiders with low cold hardiness and constant seasonal SCP, suggesting they do not fit the typical profile of a freeze-intolerant arthropod that employs physiological adaptations (e.g. cryoprotectant accumulation) to better resist freezing.

Despite this, *S. ocreata* and *S. stridulans* did not appear entirely resistant to low ambient temperatures in the leaf litter. As with other Araneae (Duman 1979), these spiders were freeze-intolerant; when cooled to -7 ˚C in the laboratory, near their mean SCP of -7.75 ˚C, no *S. stridulans* that experienced internal ice formation survived, contrasting to 47% survival of those that remained in a supercooled state. Hence, these spiders appear to be resilient to cooling in the field and are able to survive temperatures near their SCP provided the liquidity of their hemolymph is not compromised. Paradoxically, however, they were frequently at risk of freezing in the field, as temperature within the leaf litter was close to, or occasionally was below, their mean SCP (Figure 3.3). This is unusual in nature, because invertebrates typically supercool considerably below the average temperature threshold of their winter hibernacula (Somme 1982, Tanaka 1993), presumably as an adaptation to prevent high population mortality during extreme weather.

It remains unclear how *Schizocosa* overcome repeated risk of freezing during winter, while remaining a numerically dominant predator. For instance, there is no evidence that *S. ocreata* and *S. stridulans* bore into the soil for added insulation like some insects (e.g. Clarke et al. 2013); burrowing behavior has only been observed in two other species in the genus, *S. avida* (Dondale and Redner 1990), and *S. mccooki* (Suttle 2003).

Rather, they primarily seek refuge under the lowest leaf layer during sub-zero temperatures (Thomas Whitney pers. obs., George Uetz pers. comm.), a phenomenon common amongst other forest-dwelling spiders (Schaefer 1976). Although leaf litter is less insulating than subterranean retreats, it still provides reduced temperature variation and low thermal conductivity, which allows spiders to decrease their time spent in a supercooled state and thus narrows the possibility of spontaneous freezing (Edgar and Loenen 1974, Kraus and Morse, 2005). In addition, the fact that all spider species produce certain individuals with exceptionally high levels of cold hardiness may also improve winter survivorship (Kirchner 1973, Danks 1978). The positively skewed distribution that was observed in *Schizocosa* SCPs illustrates this fact (Figure 3.4). There were more exceptionally cold-hardy individuals than there were exceptionally cold-sensitive individuals, and this variation in SCP likely assists in the preservation of their high population densities. The greater question, however, is whether varying levels of winter-feeding account for these differing, albeit low, levels of cold hardiness.

A key characteristic of winter-activity is regular prey consumption, but contrary to what I hypothesized, there was no correlation between consumption and the high SCP levels observed in the laboratory. In many cases, feeding causes SCP to increase in invertebrates (e.g. Hiiesaar et al. 2009, Woodman 2012), because gut contents are an excellent source of ice nucleators (Salt 1968, Somme 1982, Bayram and Luff 1993a, Tanaka and Watanabe 1996). Interestingly, *S. ocreata* did not exhibit this trait when consuming variable quantities of prey, given that no difference in SCP was recorded (Figure 3.6). Although this is uncommon in arthropods, it has been hypothesized that SCPs in Araneae are less affected by gut contents (Salt 1961, Somme 1982, Aitchison 1987, Kirchner 1987), because spiders are fluid feeders and extra-orally digest their prey (Foelix 2011). As a consequence, they may be capable of filtering out ice nucleating agents, such as cuticular dust particles, although they still appear susceptible to bacterial ice nucleators originating in prey guts (Tanaka and Watanabe 2003). Alternatively, certain foods lack viable ice nucleators completely (Somme and Block 1982, Tanaka and Watanabe 1996), which may have been evolutionarily selected for as a means to avoid costly cryoprotectant production during winter (Duman 1991). While the exact mechanism needs further investigation, this research ultimately indicated that the amount of prey consumed had no direct effect on *Schizocosa* SCP.

There may, however, be an indirect association between foraging and cold hardiness. Given no adaptive SCP change in *S. ocreata* and *S. stridulans* occurred despite the risk of freezing, it stands to reason that their capacity to remain active and continue feeding during winter may be incompatible with accumulating cryoprotectants for increased survivorship. For example, the most common cryoprotectant found in Arthropoda is glycerol, which is highly viscous and slows hemolymph circulation, resulting in sluggish behavior (Husby and Zachariassen 1980, Zachariassen 1985). While such chemicals are widely incorporated in torpid arthropods undergoing diapause as an overwintering strategy, this would be disadvantageous for winter-active species needing to function at a higher metabolic rate (Duman 1977, Husby and Zachariassen 1980, Aitchison 1987, Bayram and Luff 1993a). Some winter-active invertebrates probably overcome this obstacle by accumulating antifreeze proteins instead of polyols (Duman 1979, Husby and Zachariassen 1980), but this is unlikely in *Schizocosa* as their SCP did not decrease in response to low temperatures. Nevertheless, this lack of cryoprotectant accumulation likely enables these spiders to actively forage and sustain steady growth throughout an unfavorable, but less competitive, time of year. This suggests an ecological tradeoff between winter survival and winter growth, where *Schizocosa* may be assuming additional risk of mortality in exchange for reproductive advantages come spring and summer, a notion previously postulated by Aitchison (1987) and Gunnarsson (1988). Both species in this study overwinter as subadults and become reproductively viable during spring (Klawinski 1996). Early maturation (Vollrath 1987, Suter 1990) and larger adult size (Kessler 1971, Vollrath 1980, Wise and Wagner 1992, Marshall and Gittleman 1994) enhances fitness through increased probability of copulation, increased number of mating opportunities, and increased fecundity. The steady winter growth that occurs in these spiders (Figure 3.5) appears necessary to achieve this timely progression into adulthood. While prey availability does increase when environmental conditions improve in the spring, competition for those resources also increase (Schaefer 1977, Kirchner 1987). Therefore, despite the potential pitfall of heightened mortality risk due to freezing, engaging in winter-foraging to avoid competition and expedite reproductive maturation appears to be advantageous for these *Schizocosa* species. Therefore, the access to prey resources during winter for timely development, rather than severe environmental stress, is more likely the greater selective pressure for these spiders.

In conclusion, I found two dominant epigeal predators in these leaf litter ecosystems, *S. ocreata* and *S. stridulans*, to have seasonally stable SCPs throughout winter, which were unaffected by prey consumption. These spiders also appeared to be faced with risk of freezing, and thus mortality, throughout this time. I speculate that they assume this risk in exchange for ability to grow during winter, as winter-activity and certain cryoprotectant accumulation are not totally compatible. Increased mating events and fecundity may compensate for increased winter mortality as a result, although further study is required to confirm this. In addition to investigating gradual acclimation to low temperatures, as I did in this study, future examination of the rapid cold hardening ability of *Schizocosa* may yield new insights into the persistence of their populations (Lee et al. 1987, Colinet and Hoffmann 2012, Teets and Denlinger 2013).

Chapter 4: Conclusions

Despite the widely held notion that spiders largely feed according to the availability of their prey, especially during winter, the results from this research provided evidence to support that they are capable of selective foraging throughout the year. From 18 months of pitfall trapping to survey the surface-active prey community, I observed availability to be positively correlated with temperature (Figure 2.5a). However, from 1231 individual *S. ocreata* and *S. stridulans* caught across the study duration and screened for prey DNA using PCR-based molecular gut-content analysis, predation frequency of Collembola and Diptera was revealed to be negatively associated with temperature and was poorly predicted by prey availability (Table 2.2). The linear food selection index (Strauss 1979) also showed evidence of preferential and non-preferential selective feeding in all seasons of the year (Figure 2.9). Moreover, in the case of Collembola and Diptera, spiders seemed to steer their feeding preferences in a balanced direction, so as to possibly maintain a diversified diet, which has been shown to improve growth in *Schizocosa* (Toft and Wise 1999). My research has displayed that not only are spiders able to express selective foraging decisions throughout the year independent of prey activity-densities, but they appear to do so in a way that optimizes fitness parameters. Furthermore, since these results were garnered from data obtained under open field conditions, rather than in an artificial laboratory environment, these findings may better reflect reality. This was one of only a few studies to examine the changes in generalist predator foraging behavior on a fully seasonal basis, and the first to do so in spiders.

Although *S. ocreata* and *S. stridulans* are found in high densities throughout the year (Appendix A), seemingly able to feed in a way that benefits fitness in individuals, the results from this study revealed these spiders likely endure a unique struggle between survival and growth with fascinating implications. From 184 spiders tested between late summer and early spring, mean SCP did not decrease (Figure 3.1) despite several occasions when litter temperatures neared or surpassed this value (Figure 3.2). This indicated that *Schizocosa* do not accumulate cryoprotectants and must endure high risk of fatal freezing, perhaps in exchange for foraging opportunities, and therefore growth opportunities, during winter. While prey consumption was found not to directly hinder SCP (Figure 3.6), the active process of foraging may indirectly increase susceptibility to freezing, since certain cryoprotectants are incompatible with winter-activity (Husby and Zachariassen 1980). These results suggest that *Schizocosa* find better success in being capable of steady winter growth than in employing physiological safeguards against low temperature mortality. This was the first study to explore the relationship between cold tolerance and winter-active feeding in spiders, and although this particular tradeoff theory is not entirely novel (Aitchison 1987, Gunnarrsson 1988), my research is the first to provide evidence compelling enough to support it.

In conclusion, *Schizocosa* spiders fed selectively during the year, including winter, where they seemed to diversify their diet presumably to maximize their growth potential. Opportunity for growth is the primary reason for winter-activity in *S. ocreata* and *S. stridulans* juveniles and is a component of their life histories proven to be effective in maintaining populations, in spite of the implicated risk of freezing. Previously, winter has been overlooked in studies of foraging behavior in generalist predators. Given that many of these studies are set in agroecosystems, where the growing season dictates the temporal range of interest, this is not a surprise. However, my study has demonstrated the importance of incorporating winter in food web analyses. This is especially true in systems with a community of winter-active predators, where winter intensity and prey populations can presumably influence reproductive success in spring. If spiders of agroecosystems make a similar tradeoff as *S. ocreata* and *S. stridulans* of Kentucky forests, for example, this information has the potential to be used in the promotion of early season biological control services, which has shown to be key in suppressing pest populations (Harwood et al. 2004, Harwood et al. 2007, Welch and Harwood 2014). In addition, since *Schizocosa* have previously been shown to greatly influence microbi-detritivore populations (Wise 2004), these results may be useful in future studies that examine the seasonal strength in cascading effects that litter-dwelling predators have on forest decomposition.

The advent of molecular techniques has made the characterization of trophic interactions easier and more accurate than ever, and the technology continues to improve. Laboratory experiments are important tools in studying ecological interactions, because variables are more easily controlled, but field studies such mine, however, are important for providing perspective to those inferences made in the laboratory. Using a multidimensional approach of field- and laboratory-based characterization of trophic linkages with traditional sampling and modern molecular techniques should prove to yield the most robust results in future foraging ecology research.

Appendices

Appendix A. Invertebrate community considered to be “potential prey” for *Schizocosa* spiders collected from pitfall traps (n = 612 over 225 days) between October 2011 and March 2013 at Berea College Forest in Madison County, Kentucky (USA).

Taxon	**Total Collected**
Total Araneae	1305
Agelenidae	39
Amaurobiidae	23
Antrodiaetidae	54
Araneidae	1
Corinnidae	58
Ctenidae	25
Dictynidae	211
Gnaphosidae	74
Linyphiidae	197
Pisauridae	3
Salticidae	15
Thomisidae	112
Total Lycosidae	497
Schizocosa spp.	493
unidentified spp.	19
Total Collembola	5637
Entomobryidae	2020
Tomoceridae	3617
Total Diplopoda	68
Total Insecta	7217
Total Archaeognatha	1
Total Blattodea	365
Total Coleoptera	587
Staphylinidae	193
unidentified larvae	394
Total Diptera	2046
Brachycera	650
Nematocera	1330
unidentified larvae	66
Total Hemiptera	17
Aphididae	3
Cicadellidae	14
Total Lepidoptera	28
unidentified larvae	28
Total Mecoptera	1
Total Orthoptera	548
Total Gryllidae	387
Allonemobius maculatus	188
Gryllus veletis	187
Rhaphidophoridae	161
Total Psocoptera	23
Total Prey	14227

Appendix B. Invertebrate community considered to be “non-potential prey” for *Schizocosa* spiders collected from pitfall traps (n = 612 over 225 days) between October 2011 and March 2013 at Berea College Forest in Madison County, Kentucky (USA).

Taxon	**Total Collected**
Total Annelida	31
Total Arthropoda	78886
Total Arachnida	1632
Total Acari	455
Total Araneae	420
Antrodiaetidae	34
Ctenizidae	1
Gnaphosidae	247
Lycosidae	60
unidentified spp.	78
Total Opiliones	737
Cosmetidae	19
Phalangiidae	718
Total Pseudoscorpionida	19
Total Scorpiones	1
Total Hexapoda	77013
Total Collembola	74371
Hypogastruridae	66776
Isotomidae	3925
Sminthuridae	3670
Total Insecta	2642
Total Coleoptera	1061
Carabidae	252
Coccinellidae	1
Curculionidae	134
Elateridae	15
Nitidulidae	268
Scarabaeidae	35
Silphidae	11
Staphylinidae	279
unidentified spp.	66
Total Hemiptera	20
Pentatomidae	1
unidentified spp.	19
Total Hymenoptera	951
Formicidae	860
Mutillidae	6
unidentified spp.	85
Total Lepidoptera	33
Total Neuroptera	2
Ascalaphidae larvae	1
unidentified larvae	1
Total Orthoptera	560
Total Caelifera	22
Total Ensifera	538
Gryllidae	385
Rhaphidophoridae	153
unidentified larvae	15
Total Myriapoda	241
Total Chilopoda	54
Total Diplopoda	187
Total Mollusca	25
Total non-prey	78942

Appendix C. Invertebrates used to assess the cross-reactivity of the general Collembola and Diptera primers and the primers specific to *Gryllus veletis*, *Allonomobius maculatus*, and *Ceuthophilus sp.* in PCR assays. All tested negative except those specifically targeted by the primers (denoted with a “+”).

Class	**Order**	**Family**	**Arthropod taxon tested**	**Collembola primers**	**Diptera primers**	**G. veletis primers**	**A. maculatus primers**	**Ceuthophilus sp. primers**
Arthropoda	Araneae		Undetermined sp.					
		Anyphaeinidae	Undetermined sp.					
		Araneidae	Undetermined sp. 1					
		Araneidae	Undetermined sp. 2					
		Araneidae	Undetermined sp. 3					
		Araneidae	Undetermined sp. 4					
		Linyphiidae	*Erigone autumnalis*					
		Linyphiidae	*Tennesseellum formica*					
		Linyphiidae	*Tennesseellum formica*					
		Linyphiidae	*Tennesseellum formica*					
		Lycosidae	Undetermined sp. 1					
		Lycosidae	Undetermined sp. 2					
		Lycosidae	*Schizocosa ocreata*					
		Lycosidae	*Schizocosa stridulans*					
		Oxyopidae	Undetermined sp. 1					
		Oxyopidae	Undetermined sp. 2					
		Salticidae	Undetermined sp. 1					
		Salticidae	Undetermined sp. 2					
		Salticidae	Undetermined sp. 3					
		Tetragnathidae	Undetermined sp.					
		Tetragnathidae	*Glenognatha foxi*					
		Tetragnathidae	*Glenognatha foxi*					
		Tetragnathidae	*Glenognatha foxi*					
		Tetragnathidae	*Glenognatha foxi*					
		Thomisidae	*Misemena sp.*					
		Thomisidae	Undetermined sp.					
	Coleoptera	Anthicidae	*Notoxus sp.*					
		Anthicidae	*Acanthinus argentinus*					
		Anthicidae	Undetermined sp.					

(continued)

Class	Order	Family	Arthropod taxon tested	Collembola primers	Diptera primers	G. veletis primers	A. maculatus primers	Ceuthophilus sp. primers
		Carabidae	*Lebia viridis*					
		Carabidae	Undetermined sp.					
		Carabidae	*Harpalus sp.* 1					
		Carabidae	*Harpalus sp.* 2					
		Chrysomelidae	*Diabrotica undecimpunctata*					
		Coccinellidae	*Coccinella septempunctata*					
		Coccinellidae	*Coleomegilla maculata*					
		Coccinellidae	*Hippodamia convergens*					
		Curculionidae	Undetermined sp.					
		Curculionidae	*Hypothenemus hampei*					
		Elateridae	Undetermined sp.					
		Latridiidae	Undetermined sp.					
		Phalacridae	Undetermined sp.					
		Staphylinidae	Undetermined sp.					
	Collembola	Tomoceridae	*Tomocerus sp.*	+				
		Entomobryidae	*Sinella curviseta*	+				
	Diptera	Chironomidae	Undetermined sp.		+			
		Chloropidae	Undetermined sp.		+			
		Dolichopodidae	Undetermined sp.		+			
		Empididae	Undetermined sp.		+			
		Ephydridae	Undetermined sp.		+			
		Heliomyzidae	Undetermined sp.		+			
		Lonochopteridae	Undetermined sp.		+			
		Muscidae	Undetermined sp.		+			
		Mycetophilidae	Undetermined sp. 1		+			
		Mycetophilidae	Undetermined sp. 2		+			
		Phoridae	Undetermined sp. 1		+			
		Phoridae	Undetermined sp. 2		+			
		Phoridae	Undetermined sp. 3		+			
		Syrphidae	Undetermined sp.		+			
		Trichoceridae	*Trichocera sp.*		+			
	Hemiptera	Aleyrodidae	Undetermined sp.					
		Alyrodidae	*Bemisia tabaci*					
		Alyrodidae	*Bemisia tabaci*					

(continued)

Class	Order	Family	Arthropod taxon tested	Collembola primers	Diptera primers	G. veletis primers	A. maculatus primers	Ceuthophilus sp. primers
		Anthocoridae	*Orius laevigatus*					
		Anthocoridae	*Orius albidipennis*					
		Pentatomidae	*Oebalus pugnax*					
		Reduviidae	Undetermined sp.					
		Rhyparachromidae	Undetermined sp.					
		Thyreocoridae	Undetermined sp.					
	Hymenoptera	Argidae	Undetermined sp.					
		Bethylidae	*Prorops nasuta*					
		Braconidae	*Aridelus*					
		Ceraphronidae	*Aphanogmus goniozi*					
		Eulophidae	*Phymastichus coffea*					
		Formicidae	Undetermined sp.					
		Formicidae	*Tapinoma sp.*					
		Platygastridae	Undetermined sp.					
		Pteromalidae	Undetermined sp.					
	Mantodea	Mantidae	Undetermined sp.					
	Opiliones	Phalangiidae	Undetermined sp.					
	Orthoptera		Undetermined sp.					
		Gryllidae	*Gryllus veletis*			+		
		Gryllidae	*Allonemobius maculatus*				+	
		Gryllidae	Undetermined sp.					
		Rhaphidophoridae	*Ceuthophilus sp.*					+
		Tettigoniidae	Undetermined sp.					
	Psocoptera	Psocoptera	Undetermined sp.					
	Thysanoptera	Phlaeothripidae	*Karnyothrips flavipes*					
		Thripidae	*Frankliniella occidentalis*					
	Trombidoformes		Undetermined sp.					
		Trombidiidae	Undetermined sp.					
Mollusca	Helicoidea	Polygyridae	*Mesodon zaletus*					
	Punctoidea	Discidae	*Aniguispira alternata*					

References

Aitchison, C. W. 1984a. Low-temperature feeding by winter-active spiders. Journal of Arachnology **12**:297-305.

Aitchison, C. W. 1984b. The phenology of winter-active spiders. Journal of Arachnology **12**:249-271.

Aitchison, C. W. 1987. Feeding ecology of winter-active spiders. Pages 264-273 *in* W. Nentwig, editor. Ecophysiology of Spiders. Springer-Verlag, Berlin, Germany.

Anderson, J. F. 1970. Metabolic rates of spiders. Comparative Biochemistry and Physiology **33**:51-72.

Anderson, J. F. 1974. Responses to starvation in spiders *Lycosa lenta* Hentz and *Filistata hibernalis* (Hentz). Ecology **55**:576-585.

Anderson, J. F., and K. N. Prestwich. 1982. Respiratory gas-exchange in spiders. Physiological Zoology **55**:72-90.

Bale, J. S. 2002. Insects and low temperatures: from molecular biology to distributions and abundance. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **357**:849-861.

Bardwell, C. J., and A. L. Averill. 1997. Spiders and their prey in Massachusetts cranberry bogs. Journal of Arachnology **25**:31-41.

Bayram, A., and M. L. Luff. 1993a. Cold-hardiness of wolf spiders (Lycosidae, Araneae) with particular reference to *Pardosa pullata* (Clerck). Journal of Thermal Biology **18**:263-268.

Bayram, A., and M. L. Luff. 1993b. Winter abundance and diversity of lycosids (Lycosidae, Araneae) and other spiders in grass tussocks in a field margin. Pedobiologia **37**:357-364.

Begg, C. M., K. S. Begg, J. T. Du Toit, and M. G. L. Mills. 2003. Sexual and seasonal variation in the diet and foraging behaviour of a sexually dimorphic carnivore, the honey badger (*Mellivora capensis*). Journal of Zoology **260**:301-316.

Behmer, S. T. 2009. Insect herbivore nutrient regulation. Annual Review of Entomology **54**:165-187.

Bilde, T., J. A. Axelsen, and S. Toft. 2000. The value of Collembola from agricultural soils as food for a generalist predator. Journal of Applied Ecology **37**:672-683.

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

Bitzer, C., G. Brasse, K. Dettner, and S. Schulz. 2004. Benzoic acid derivatives in a Hypogastrurid collembolan: Temperature-dependent formation and biological significance as deterrents. Journal of Chemical Ecology **30**:1591-1602.

Block, W., and J. Zettel. 1980. Cold hardiness of some alpine Collembola. Ecological Entomology **5**:1-9.

Block, W., and J. Zettel. 2003. Activity and dormancy in relation to body water and cold tolerance in a winter-active springtail (Collembola). European Journal of Entomology **100**:305-312.

Boiteau, G., and P. MacKinley. 2012. Locomotor response of *Folsomia candida* (Collembola: Isotomidae) to cooling temperatures. Environmental Entomology **41**:916-924.

Castro, A., and D. H. Wise. 2009. Influence of fine woody debris on spider diversity and community structure in forest leaf litter. Biodiversity and Conservation **18**:3705-3731.

Castro, A., and D. H. Wise. 2010. Influence of fallen coarse woody debris on the diversity and community structure of forest-floor spiders (Arachnida: Araneae). Forest Ecology and Management **260**:2088-2101.

Catley, K. M. 1992. Supercooling and its ecological implications in *Coelotes atropos* (Araneae, Agelenidae). Journal of Arachnology **20**:58-63.

Chapman, E. G., J. M. Schmidt, K. D. Welch, and J. D. Harwood. 2013. Molecular evidence for dietary selectivity and pest suppression potential in an epigeal spider community in winter wheat. Biological Control **65**:72-86.

Chen, B. R., and D. H. Wise. 1999. Bottom-up limitation of predaceous arthropods in a detritus-based terrestrial food web. Ecology **80**:761-772.

Clark, D. L., J. A. Roberts, M. Rector, and G. W. Uetz. 2011. Spectral reflectance and communication in the wolf spider, *Schizocosa ocreata* (Hentz): simultaneous crypsis and background contrast in visual signals. Behavioral Ecology and Sociobiology **65**:1237-1247.

Clarke, M. W., G. J. Thompson, and B. J. Sinclair. 2013. Cold Tolerance of the Eastern Subterranean Termite, *Reticulitermes flavipes* (Isoptera: Rhinotermitidae), in Ontario. Environmental Entomology **42**:805-810.

Colinet, H., and A. A. Hoffmann. 2012. Comparing phenotypic effects and molecular correlates of developmental, gradual and rapid cold acclimation responses in Drosophila melanogaster. Functional Ecology **26**:84-93.

Crosthwaite, J. C., S. Sobek, D. B. Lyons, M. A. Bernards, and B. J. Sinclair. 2011. The overwintering physiology of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). Journal of Insect Physiology **57**:166-173.

Danks, H. V. 1978. Modes of seasonal adaptation in insects: I. Winter survival. Canadian Entomologist **110**:1167-1205.

Dondale, C. D., and J. H. Redner. 1990. The Wolf Spiders, Nurseryweb Spiders, and Lynx Spiders of Canada and Alaska. Biosystematics Research Centre, Ottawa, ON, Canada.

Doucet, D., V. K. Walker, and W. Qin. 2009. The bugs that came in from the cold: molecular adaptations to low temperatures in insects. Cellular and Molecular Life Sciences **66**:1404-1418.

Duman, J. G. 1977. Role of macromolecular antifreeze in darkling beetle, *Meracantha contracta*. Journal of Comparative Physiology **115**:279-286.

Duman, J. G. 1979. Subzero temperature tolerance in spiders: Role of thermal-hysteresis factors. Journal of Comparative Physiology **131**:347-352.

Duman, J. G. 2001. Antifreeze and ice nucleator proteins in terrestrial arthropods. Annual Review of Physiology **63**:327-357.

Duman, J. G., D. W. Wu, L. Xu, D. Tursman, and T. M. Olsen. 1991. Adaptations of insects to subzero temperatures. Quarterly Review of Biology **66**:387-410.

Edgar, W., and M. Loenen. 1974. Aspects of overwintering habitat of wolf spider *Pardosa lugubris*. Journal of Zoology **172**:383-388.

Ehler, L. E. 1977. Natural enemies of cabbage looper (Lepidoptera: Noctuidae) on cotton in San Joaquin Valley. Hilgardia **45**:73-106.

Eitzinger, B., A. Micic, M. Korner, M. Traugott, and S. Scheu. 2013. Unveiling soil food web links: New PCR assays for detection of prey DNA in the gut of soil arthropod predators. Soil Biology & Biochemistry **57**:943-945.

Eitzinger, B., and M. Traugott. 2011. Which prey sustains cold-adapted invertebrate generalist predators in arable land? Examining prey choices by molecular gut-content analysis. Journal of Applied Ecology **48**:591-599.

Eubanks, M. D., and R. F. Denno. 2000. Health food versus fast food: the effects of prey quality and mobility on prey selection by a generalist predator and indirect interactions among prey species. Ecological Entomology **25**:140-146.

Fantinou, A. A., D. C. Perdikis, P. D. Labropoulos, and D. A. Maselou. 2009. Preference and consumption of *Macrolophus pygmaeus* preying on mixed instar assemblages of *Myzus persicae*. Biological Control **51**:76-80.

Finke, D. L., and R. F. Denno. 2004. Predator diversity dampens trophic cascades. Nature **429**:407-410.

Foelix, R. 2011. Biology of spiders. 3rd edition. Oxford University Press, New York, USA.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome coxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology **3**:294-299.

Galef, B. G. 1996. Food selection: Problems in understanding how we choose foods to eat. Neuroscience and Biobehavioral Reviews **20**:67-73.

Gillespie, R. G., and T. Caraco. 1987. Risk-sensitive foraging strategies of 2 spider populations. Ecology **68**:887-899.

Gray, R. D. 1987. Faith and Foraging: A Critique of the "Paradigm Argument from Design". Pages 69-140 *in* A. C. Kamil, J. R. Krebs, and H. R. Pulliam, editors. Foraging Ecology. Springer US.

Grear, J. S., and O. J. Schmitz. 2005. Effects of grouping behavior and predators on the spatial distribution of a forest floor arthropod. Ecology **86**:960-971.

Greenstone, M. H. 1979. Spider feeding behavior optimizes dietary essential amino acid composition. Nature **282**:501-503.

Greenstone, M. H., and A. F. Bennett. 1980. Foraging strategy and metabolic rate in spiders. Ecology **61**:1255-1259.

Gunnarsson, B. 1985. Interspecific predation as a mortality factor among overwintering spiders. Oecologia **65**:498-502.

Gunnarsson, B. 1988. Body size and survival: Implications for an overwintering spider. Oikos **52**:274-282.

Harwood, J. D., N. Desneux, H. J. S. Yoo, D. L. Rowley, M. H. Greenstone, J. J. Obrycki, and R. J. O'Neil. 2007. Tracking the role of alternative prey in soybean aphid predation by *Orius insidiosus*: a molecular approach. Molecular Ecology **16**:4390-4400.

Harwood, J. D., S. W. Phillips, J. Lello, K. D. Sunderland, D. M. Glen, M. W. Bruford, G. L. Harper, and W. O. C. Symondson. 2009. Invertebrate biodiversity affects predator fitness and hence potential to control pests in crops. Biological Control **51**:499-506.

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

Harwood, J. D., K. D. Sunderland, and W. O. C. Symondson. 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.

Hebert, P. D. N., A. Cywinska, S. L. Ball, and J. R. DeWaard. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society B-Biological Sciences **270**:313-321.

Hiiesaar, K., I. Williams, A. Luik, L. Metspalu, R. Muljar, K. Jogar, R. Karise, M. Mand, E. Svilponis, and A. Ploomi. 2009. Factors affecting cold hardiness in the small striped flea beetle, *Phyllotreta undulata*. Entomologia Experimentalis Et Applicata **131**:278-285.

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

Hoogendoorn, M., and G. E. Heimpel. 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.

Hopkin, S. P. 1997. Biology of the Springtails. Oxford University Press, Oxford, UK.

Huhta, V., and J. Viramo. 1979. Spiders active on snow in Northern Finland. Annales Zoologici Fennici **16**:169-176.

Husby, J. A., and K. E. Zachariassen. 1980. Antifreeze agents in the body fluid of winter-active insects and spiders. Experientia **36**:963-964.

Ishijima, C., A. Taguchi, M. Takagi, T. Motobayashi, M. Nakai, and Y. Kunimi. 2006. Observational evidence that the diet of wolf spiders (Araneae : Lycosidae) in paddies temporarily depends on dipterous insects. Applied Entomology and Zoology **41**:195-200.

Jackson, R. R., X. J. Nelson, and G. O. Sune. 2005. A spider that feeds indirectly on vertebrate blood by choosing female mosquitoes as prey. Proceedings of the National Academy of Sciences of the United States of America **102**:15155-15160.

Jaskula, R., and A. Soszynska-Maj. 2011. What do we know about winter active ground beetles (Coleoptera, Carabidae) in Central and Northern Europe? Zookeys **100**:517-532.

Jensen, K., D. Mayntz, S. Toft, F. J. Clissold, J. Hunt, D. Raubenheimer, and S. J. Simpson. 2012. Optimal foraging for specific nutrients in predatory beetles. Proceedings of the Royal Society B-Biological Sciences **279**:2212-2218.

Jensen, K., D. Mayntz, S. Toft, D. Raubenheimer, and S. J. Simpson. 2011. Nutrient regulation in a predator, the wolf spider *Pardosa prativaga*. Animal Behaviour **81**:993-999.

Juen, A., K. H. Steinberger, and M. Traugott. 2003. Seasonal change in species composition and size distribution of epigeic predators in a small field. Entomologia Generalis **26**:259-275.

Kamil, A. C., J. R. Krebs, and H. R. Pulliam. 1987. Foraging Behavior. New York: Plenum Press, New York, NY, USA.

Karlin, S., and S. F. Altschul. 1990. Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes. Proceedings of the National Academy of Sciences of the United States of America **87**:2264-2268.

Kato, C., T. Iwata, S. Nakano, and D. Kishi. 2003. Dynamics of aquatic insect flux affects distribution of riparian web-building spiders. Oikos **103**:113-120.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, and A. Drummond. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics **28**:1647-1649.

Kessler, A. 1971. Relation between egg production and food consumption in species of genus Pardosa (Araneae: Lycosidae) under experimental conditions of food-abundance and food-shortage. Oecologia **8**:93-109.

Khodayari, S., H. Colinet, S. Moharramipour, and D. Renault. 2013. Seasonal changes in the cold hardiness of the two-spotted spider mite females (Acari: Tetranychidae). Environmental Entomology **42**:1415-1421.

Kirchner, W. 1973. Ecological aspects of cold resistance in spiders (A comparative study). Pages 271-279 *in* W. Weiser, editor. Effect of Temperature on Ectothermic Organisms. Springer Berlin Heidelberg, New York, USA.

Kirchner, W. 1987. Behavioural and Physiological Adaptations to Cold. Pages 66-77 *in* W. Nentwig, editor. Ecophysiology of Spiders. Springer-Verlag, Berlin, Germany.

Kirchner, W., and P. Kestler. 1969. Cold resistance of *Araneus cornutus* (Araneidae) spiders. Journal of Insect Physiology **15**:41-53.

Klawinski, P. D. 1996. Foraging, development and life history of the wolf spider, *Schizocosa ocreata*. Dissertation. University of Texas at Arlington, Ann Arbor, MI, USA.

Kobayashi, T., M. Takada, S. Takagi, A. Yoshioka, and I. Washitani. 2011. Spider predation on a mind pest in Japanese rice fields. Basic and Applied Ecology **12**:532-539.

Korenko, S., and S. Pekar. 2010. Is there intraguild predation between winter-active spiders (Araneae) on apple tree bark? Biological Control **54**:206-212.

Korenko, S., S. Pekar, and A. Honek. 2010. Predation activity of two winter-active spiders (Araneae: Anyphaenidae, Philodromidae). Journal of Thermal Biology **35**:112-116.

Kostal, V., P. Simek, H. Zahradnickova, J. Cimlova, and T. Stetina. 2012. Conversion of the chill susceptible fruit fly larva (Drosophila melanogaster) to a freeze tolerant organism. Proceedings of the National Academy of Sciences of the United States of America **109**:3270-3274.

Kraus, J. M., and D. H. Morse. 2005. Seasonal habitat shift in an intertidal wolf spider: Proximal cues associated with migration and substrate preference. Journal of Arachnology **33**:110-123.

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

Kuusk, A. K., and N. Agusti. 2008. Group-specific primers for DNA-based detection of springtails (Hexapoda: Collembola) within predator gut contents. Molecular Ecology Resources **8**:678-681.

Kuusk, A. K., and B. Ekbom. 2012. Feeding habits of lycosid spiders in field habitats. Journal of Pest Science **85**:253-260.

Lee, K. P., S. J. Simpson, F. J. Clissold, R. Brooks, J. W. O. Ballard, P. W. Taylor, N. Soran, and D. Raubenheimer. 2008. Lifespan and reproduction in Drosophila: New insights from nutritional geometry. Proceedings of the National Academy of Sciences of the United States of America **105**:2498-2503.

Lee, R. E. 1989. Insect cold-hardiness: To freeze or not to freeze. Bioscience **39**:308-313.

Lee, R. E., C. P. Chen, and D. L. Denlinger. 1987. A rapid cold-hardening process in insects. Science **238**:1415-1417.

Lee, R. E., Jr. 2010. A primer on insect cold-tolerance. Pages 3-34 *in* D. L. Denlinger and R. E. Lee, Jr., editors. Low temperature biology of insects. Cambridge University Press.

Lensing, J. R., and D. H. Wise. 2006. Predicted climate change alters the indirect effect of predators on an ecosystem process. Proceedings of the National Academy of Sciences of the United States of America **103**:15502-15505.

Lundheim, R. 2002. Physiological and ecological significance of biological ice nucleators. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **357**:937-943.

Macarthur, R. H., and E. R. Pianka. 1966. On optimal use of a patchy environment. American Naturalist **100**:603-609.

Magura, T., B. Tothmeresz, and Z. Elek. 2004. Effects of leaf-litter addition on carabid beetles in a non-native Norway spruce plantation. Acta Zoologica Academiae Scientiarum Hungaricae **50**:9-23.

Marcussen, B. M., J. A. Axelsen, and S. Toft. 1999. The value of two Collembola species as food for a linyphiid spider. Entomologia Experimentalis Et Applicata **92**:29-36.

Marden, J. H. 1989. Bodybuilding dragonflies: Costs and benefits of maximizing flight muscle. Physiological Zoology **62**:505-521.

Marshall, S. D., and J. L. Gittleman. 1994. Clutch size in spiders: Is more better? Functional Ecology **8**:118-124.

Mayntz, D., D. Raubenheimer, M. Salomon, S. Toft, and S. J. Simpson. 2005. Nutrient-specific foraging in invertebrate predators. Science **307**:111-113.

Mayntz, D., and S. Toft. 2001. Nutrient composition of the prey's diet affects growth and survivorship of a generalist predator. Oecologia **127**:207-213.

McLachlan, A. J., and R. M. Neems. 1996. Flight architecture determined by physical constraints or by natural selection?: The case of the midge Chironomus plumosus. Journal of Zoology **240**:301-308.

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

Morse, D. H. 1997. Distribution, movement, and activity patterns of an intertidal wolf spider *Pardosa lapidicina* population (Araneae: Lycosidae). Journal of Arachnology **25**:1-10.

Moulder, B. C., and D. E. Reichle. 1972. Significance of spider predation in energy dynamics of forest-floor arthropod communities. Ecological Monographs **42**:473-498.

Moya-Larano, J., and D. H. Wise. 2007. Direct and indirect effects of ants on a forest-floor food web. Ecology **88**:1454-1465.

Murdoch, W. W. 1969. Switching in general predators: experiments on predator specificity and stability of prey populations. Ecological Monographs.

Nentwig, W. 1982. Epigenic spiders, their potential prey and competitors: Relationship between size and frequency. Oecologia **55**:130-136.

Nentwig, W. 1986. Non-webbuilding spiders: prey specialists or generalists? Oecologia **69**:571-576.

Nentwig, W. 1987. The Prey of Spiders. Pages 249-263 *in* W. Nentwig, editor. Ecophysiology of Spiders. Springer-Verlag, Berlin.

Nentwig, W., and C. Wissel. 1986. A comparison of prey lengths among spiders. Oecologia **68**:595-600.

Oelbermann, K., R. Langel, and S. Scheu. 2008. Utilization of prey from the decomposer system by generalist predators of grassland. Oecologia **155**:605-617.

Perry, G., and E. R. Pianka. 1997. Animal foraging: past, present and future. Trends in Ecology & Evolution **12**:360-364.

Peterson, J. A., S. A. Romero, and J. D. Harwood. 2010. Pollen interception by linyphiid spiders in a corn agroecosystem: implications for dietary diversification and risk-assessment. Arthropod-Plant Interactions **4**:207-217.

Pollet, M., and K. Desender. 1987. Feeding ecology of grassland-inhabiting carabid beetles (Coleoptera: Carabidae) in relation to the availability of some prey groups. Acta Phytopathologica Et Entomologica Hungarica **22**:223-246.

Ratnasingham, S., and P. D. N. Hebert. 2007. BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). Molecular Ecology Notes **7**:355-364.

Rickers, S., R. Langel, and S. Scheu. 2006. Dietary routing of nutrients from prey to offspring in a generalist predator: effects of prey quality. Functional Ecology **20**:124-131.

Riechert, S. E. 1991. Prey abundance vs diet breadth in a spider test system. Evolutionary Ecology **5**:327-338.

Riechert, S. E., and L. Bishop. 1990. Prey control by an assemblage of generalist predators: spiders in garden test systems. Ecology **71**:1441-1450.

Riechert, S. E., and J. M. Harp. 1987. Nutritional ecology of spiders. Pages 645-672 *in* F. Slansky and J. G. Rodriguez, editors. Nutritional Ecology of Insects, Mites and Spiders. Wiley, New York, NY, USA.

Riechert, S. E., and K. Lawrence. 1997. Test for predation effects of single versus multiple species of generalist predators: spiders and their insect prey. Entomologia Experimentalis Et Applicata **84**:147-155.

Romero, S. A., and J. D. Harwood. 2010. Diel and seasonal patterns of prey available to epigeal predators: Evidence for food limitation in a linyphiid spider community. Biological Control **52**:84-90.

Rypstra, A. L., and S. D. Marshall. 2005. Augmentation of soil detritus affects the spider community and herbivory in a soybean agroecosystem. Entomologia Experimentalis Et Applicata **116**:149-157.

Salt, R. W. 1961. Principles of insect cold-hardiness. Annual Review of Entomology **6**:55-73.

Salt, R. W. 1968. Location and quantitative aspects of ice nucleators in insects. Canadian Journal of Zoology **46**:329-333.

Samu, F., and Z. Biro. 1993. Functional response, multiple feeding and wasteful killing in a wolf spider (Araneae: Lycosidae). European Journal of Entomology **90**:471-476.

Schaefer, M. 1976. Experimentelle Untersuchungen zum Jahreszyklus und zur Uberwinterung von Spinnen (Araneida). Zoologische Jb (Syst) **103**:127-289.

Schaefer, M. 1977. Winter ecology of spiders (Araneida). Zeitschrift Fur Angewandte Entomologie-Journal of Applied Entomology **83**:113-134.

Scharf, I., E. Nulman, O. Ovadia, and A. Bouskila. 2006. Efficiency evaluation of two competing foraging modes under different conditions. American Naturalist **168**:350-357.

Schmidt, J. M., J. D. Harwood, and A. L. Rypstra. 2012. Foraging activity of a dominant epigeal predator: molecular evidence for the effect of prey density on consumption. Oikos **121**:1715-1724.

Schmidt, J. M., J. A. Peterson, J. G. Lundgren, and J. D. Harwood. 2013. Dietary supplementation with pollen enhances survival and Collembola boosts fitness of a web-building spider. Entomologia Experimentalis Et Applicata **149**:282-291.

Schmidt-Entling, M. H., and E. Siegenthaler. 2009. Herbivore release through cascading risk effects. Biology Letters **5**:773-776.

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

Shultz, B. J., J. R. Lensing, and D. H. Wise. 2006. Effects of altered precipitation and wolf spiders on the density and activity of forest-floor Collembola. Pedobiologia **50**:43-50.

Sinclair, B. J., P. Vernon, C. J. Klok, and S. L. Chown. 2003. Insects at low temperatures: an ecological perspective. Trends in Ecology & Evolution **18**:257-262.

Sint, D., L. Raso, and M. Traugott. 2012. Advances in multiplex PCR: balancing primer efficiencies and improving detection success. Methods in Ecology and Evolution **3**:898-905.

Slansky, F., and J. M. Scriber. 1985. Food consumption and utilization. Pages 87-163 *in* G. A. Kerkut, editor. Comprehensive insect physiology, biochemistry and pharmacology 4. Pergamon.

Somme, L. 1982. Supercooling and winter survival in terrestrial arthropods. Comparative Biochemistry and Physiology a-Physiology **73**:519-543.

Somme, L., and W. Block. 1982. Cold hardiness of Collembola at Signy Island, Maritime Antarctic. Oikos **38**:168-176.

Stephens, D. W., and J. R. Krebs. 1986. Foraging Theory. Princeton University Press, Princeton, NJ, USA.

Storey, K. B., and J. M. Storey. 2012. Insect cold hardiness: metabolic, gene, and protein adaptation. Canadian Journal of Zoology-Revue Canadienne De Zoologie **90**:456-475.

Stratton, G. E. 1991. A new species of wolf spider, *Schizocosa stridulans* (Araneae: Lycosidae). Journal of Arachnology **19**:29-39.

Strauss, R. E. 1979. Reliability estimates for Ivlev electivity index, the forage ratio, and a proposed linear index of food selection. Transactions of the American Fisheries Society **108**:344-352.

Suter, R. B. 1990. Courtship and the assessment of virginity by male bowl and doily spiders. Animal Behaviour **39**:307-313.

Suttle, K. B. 2003. Burrow use in a northern California population of the wolf spider *Schizocosa mccooki* (Araneae, Lycosidae). Journal of Arachnology **31**:433-436.

Swift, M. J., O. W. Heal, and J. M. Anderson. 1979. Decomposition in terrestrial ecosystems. Blackwell Scientific Publishers, Oxford.

Symondson, W. O. C. 2002. Molecular identification of prey in predator diets. Molecular Ecology **11**:627-641.

Symondson, W. O. C., K. D. Sunderland, and M. H. Greenstone. 2002. Can generalist predators be effective biocontrol agents? Annual Review of Entomology **47**:561-594.

Tanaka, K. 1993. Seasonal change in cold tolerance of the house spider, *Achaearanea tepidariorum* (Araneae: Theridiidae). Acta arachnologica **42**:151-158.

Tanaka, K., and M. Watanabe. 1996. Influence of prey species on the supercooling ability of the Redback Spider, *Latrodectus hasseltii* (Araneae: Theridiidae). Acta arachnologica **45**:147-150.

Tanaka, K., and M. Watanabe. 2003. Transmission of ice-nucleating active bacteria from a prey reduces cold hardiness of a predator (Araneae : Theridiidae). Naturwissenschaften **90**:449-451.

Teets, N. M., and D. L. Denlinger. 2013. Physiological mechanisms of seasonal and rapid cold-hardening in insects. Physiological Entomology **38**:105-116.

Toft, S. 1995. Value of the aphid *Rhopalosiphum padi* as food for cereal spiders. Journal of Applied Ecology **32**:552-560.

Toft, S., and D. H. Wise. 1999. Growth, development, and survival of a generalist predator fed single- and mixed-species diets of different quality. Oecologia **119**:191-197.

Tschanz, B., L. F. Bersier, and S. Bacher. 2007. Functional responses: A question of alternative prey and predator density. Ecology **88**:1300-1308.

Uetz, G. W. 1992. Foraging strategies of spiders. Trends in Ecology & Evolution **7**:155-159.

Uetz, G. W. 1999. Guild structure of spiders in major crops. Journal of Arachnology **27**:270-280.

Uetz, G. W., and J. A. Roberts. 2002. Multisensory cues and multimodal communication in spiders: Insights from video/audio playback studies. Brain Behavior and Evolution **59**:222-230.

Uetz, G. W., J. A. Roberts, D. L. Clark, J. S. Gibson, and S. D. Gordon. 2013. Multimodal signals increase active space of communication by wolf spiders in a complex litter environment. Behavioral Ecology and Sociobiology **67**:1471-1482.

Vanin, S., L. Bubacco, and M. Beltramini. 2008. Seasonal variation of trehalose and glycerol concentrations in winter snow-active insects. Cryoletters **29**:485-491.

Vanin, S., and M. Turchetto. 2007. Winter activity of spiders and pseudoscorpions in the South Eastern Alps (Italy). Italian Journal of Zoology **74**:31-38.

Venner, S., and J. Casas. 2005. Spider webs designed for rare but life-saving catches. Proceedings of the Royal Society B-Biological Sciences **272**:1587-1592.

Vollrath, F. 1980. Male body size and fitness in the web-building spider *Nephila clavipes*. Zeitschrift Fur Tierpsychologie-Journal of Comparative Ethology **53**:61-78.

Vollrath, F. 1987. Growth, Foraging and Reproductive Success. Pages 357-270 *in* W. Nentwig, editor. Ecophysiology of Spiders. Springer-Verlag, Berlin.

von Berg, K., M. Traugott, W. O. C. Symondson, and S. Scheu. 2008. The effects of temperature on detection of prey DNA in two species of carabid beetle. Bulletin of Entomological Research **98**:263-269.

Wagner, J. D., and D. H. Wise. 1997. Influence of prey availability and conspecifics on patch quality for a cannibalistic forager: Laboratory experiments with the wolf spider *Schizocosa*. Oecologia **109**:474-482.

Watanabe, M. 2002. Cold tolerance and myo-inositol accumulation in overwintering adults of a lady beetle, *Harmonia axyridis* (Coleoptera : Coccinellidae). European Journal of Entomology **99**:5-9.

Welch, K. D., and J. D. Harwood. 2014. Temporal dynamics of natural enemy-pest interactions in a changing environment. Biological Control **In press**.

Welch, K. D., K. F. Haynes, and J. D. Harwood. 2013. Prey-specific foraging tactics in a web-building spider. Agricultural and Forest Entomology **15**:375-381.

Wilder, S. M. 2011. Spider Nutrition: An Integrative Perspective. Pages 87-136 Advances in Insect Physiology, Vol 40: Spider Physiology and Behaviour - Physiology. Academic Press Ltd-Elsevier Science Ltd, London.

Wilder, S. M., and A. L. Rypstra. 2010. Males make poor meals: a comparison of nutrient extraction during sexual cannibalism and predation. Oecologia **162**:617-625.

Williams, D. D. 1987. A Laboratory Study of Predator Prey Interactions of Stoneflies and Mayflies. Freshwater Biology **17**:471-490.

Wise, D. H. 1993. Spiders in ecological webs. Cambridge University Press, Cambridge.

Wise, D. H. 2004. Wandering spiders limit densities of a major microbi-detritivore in the forest-floor food web. Pedobiologia **48**:181-188.

Wise, D. H. 2006. Cannibalism,food limitation, intraspecific competition and the regulation of spider populations. Pages 441-465 Annual Review of Entomology. Annual Reviews, Palo Alto.

Wise, D. H., and J. D. Wagner. 1992. Evidence of exploitative competition among young stages of the wolf spider *Schizocosa ocreata*. Oecologia **91**:7-13.

Woodman, J. D. 2012. Cold tolerance of the Australian spur-throated locust, *Austracris guttulosa*. Journal of Insect Physiology **58**:384-390.

Zachariassen, K. E. 1985. Physiology of cold tolerance in insects. Physiological Reviews **65**:799-832.

Vita: Thomas Edward Dantas Whitney

Education

B.S., Biology, concentration Environmental Studies
Gonzaga University, Spokane, WA.
Completed: May 2011.

Relevant Positions

Graduate Research Assistant, Invertebrate Ecology Lab (Dr. JD Harwood)
University of Kentucky, Department of Entomology
July 2011-present

HHMI Research Fellow, Insect Ecology Lab (Dr. GC Chang)
Gonzaga University, Biology Department
Project title: *Does social feeding improve larval survival of the two-spotted lady beetle (Adalia bipunctata)?*
September 2010-May 2011

Research Science Aid, Entomology Lab (Dr. JC Lee)
USDA ARS, Corvallis, OR
Summers of 2008, 2009, and 2010

Study Abroad Directed Research Student, Tropical Ecology (Dr. E Arévalo)
School for Field Studies, Costa Rica
Project title: *Impacts of road disturbances on diversity and abundance of ground-occurring insects*
September 2009-December 2009

Fellowships

- National Science Foundation Graduate Research Fellowship, August 2011-present ($32,500 + tuition per year).
- Howard Hughes Medical Institute Research Fellowship, Gonzaga University, 2010-2011 ($1,500).
- Gonzaga Regent's Merit Scholarship, Francis W Conn SJ Memorial Scholarship, Lawrence E Duffy Endowed Grant, and Gonzaga Funding, 2007-2011 ($60,000).

Awards

- 1st Place, Student Competition for the President's Prize (Ten-Minute Paper, Section P-IE21), Annual Meeting of the Entomological Society of America 2013, Austin, TX ($175).

Publications: Peer Reviewed

Whitney TD, Philip BN & JD Harwood. Tradeoff in two winter-active wolf spiders: increased mortality for increased growth. Entomologia Experimentalis et Applicata. In review.

Moore M, Burt C, **Whitney TD**, Hastings S & G Chang. 2012. Does social feeding mitigate larval starvation in two spotted lady beetles (*Adalia bipunctata*)? The Journal of Insect Science 12:101.

Publications: Extension

Walton V, J Lee, D Bruck, P Shearer, E Parent, **TD Whitney** & A Dreves. 2010. Recognize fruit damage from spotted wing Drosophila (SWD), *Drosophila suzukii*. Oregon State University Extension, EM 9021, 4 pp. http://ir.library.oregonstate.edu/xmlui/handle/1957/19525

Parent E, **Whitney TD** & JC Lee. 2010. Recognizing fruit damaged by spotted wing Drosophila. USDA ARS, 2 pp. http://ars.usda.gov/pandp/docs.htm?docid=17567

Oral Presentations

Whitney TD & JD Harwood. Nov 2013. Tracking temporal predation shifts in forest wolf spiders. Annual Meeting of the Entomological Society of America, Austin, TX.

Whitney TD & JD Harwood. Aug 2013. Tracking temporal predation shifts in forest-dwelling spiders. Congress for the International Association of Ecology, London, UK.

Whitney TD, Philip BN & JD Harwood. July 2013. The effects of season and prey consumption on wolf spider cold tolerance. Annual Meeting of the American Arachnalogical Society, Johnson City, TN.

Whitney TD & JD Harwood. May 2013. Prey availability and its effects on seasonal predation patterns in forest-dwelling spiders. The 2nd International Symposium on the Molecular Detection of Trophic Interactions, Lexington, KY.

Whitney TD & JD Harwood. May 2013. Prey availability and its effects on seasonal predation patterns in forest-dwelling spiders. Annual UK Center for Ecology, Evolution, and Behavior Spring Symposium, Lexington, KY.

Whitney TD & JD Harwood. Apr 2013. Seasonal variation in trophic strength between a litter-dwelling spider and its favorite prey. Annual Meeting of the Forest Resources and Environmental Conservation department, Blacksburg, VA.

Whitney TD & JD Harwood. Nov 2012. Temporal shifts in spider foraging within a forest foodweb. Annual Meeting of the Entomological Society of America, Knoxville, TN.

Poster Presentations

Philip BN, **Whitney TD** & JD Harwood. Aug 2013. Seasonal stability of supercooling points in spiders living in a temperate ecosystem. Fifth International Symposium on the Environmental Physiology of Ectotherms and Plants, London, ON, Canada.

Whitney, TD & JD Harwood. Aug 2012. Tracking predation shifts in forest spiders during the cold season. Annual Meeting of the Ecological Society of America, Portland, OR.

Whitney, TD & JD Harwood. Jul 2012. Characterizing the changes in forest spider feeding during the cold season. Annual Meeting of the American Arachnalogical Society, Green Bay, WI.

Whitney TD, Moore M, Burt C, Hastings S, & G Chang. Nov 2011. Does social feeding mitigate larval starvation in two spotted lady beetles (*Adalia bipunctata*)? Annual Meeting of the Entomological Society of America, Reno, NV.

Harwood JD, Allen CD, Chapman EG, Johansen KJ, Kowles KA, McKenrick HJ, Peterson JA, Schmidt JM, Welch KD & **TD Whitney**. 2011. Disentangling the spider's web: insights from complex terrestrial food webs. Annual Meeting of the ESA, Reno, NV.

Whitney TD. Nov 2010. Impacts of road disturbances on diversity and abundance of ground-occurring insects. Murdock College Science Research Conference, McMinnville, OR.

Whitney TD. Oct 2010. Impacts of road disturbances on diversity and abundance of ground-occurring insects. Gonzaga Science Research Program Fall Family Weekend Poster Session, Spokane, WA.

Teaching

Insect Biology (Entomology 110), University of Kentucky
Teaching Assistant, January - May 2013, enrollment = 80
Presented lecture on insect adaptations to extreme environmental conditions, including diapause and overwintering strategies. Provided assistance with set-up of daily class activities, assisted students with in-class assignments, gave insect identifications for student collections, and graded daily assignments, quizzes and exams.

Professional Affiliations

Ecological Society of America
Entomological Society of America
American Arachnological Society
British Ecological Society

Reviewing and Editorial

Reviewer for:
Molecular Ecology