THE ROLE OF SPIDERS IN THE DETRITAL FOOD WEB OF AN EASTERN DECIDUOUS FOREST

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THE ROLE OF SPIDERS IN THE DETRITAL FOOD WEB
OF AN EASTERN DECIDUOUS FOREST

ABSTRACT OF DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Agriculture at the University of Kentucky

By

Erin Elizabeth Hladilek

Lexington, KY

Co-directors: Dr. Mary Arthur, Professor of Forestry and Dr. John Obrycki, Professor of Entomology

Lexington, KY

2009

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ABSTRACT OF DISSERTATION

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Historically, terrestrial food web research has focused on describing the structure of aboveground grazing webs, and determining how interactions among plants, herbivores and higher trophic levels influence primary productivity. Detrital food webs however, play a significant role in regulation of ecosystem dynamics through direct impacts on decomposition. Unraveling the complex nature of detrital food web structure is critical to developing a better understanding of ecosystem function. Therefore the primary objective of this research was to describe the structure of the leaf-litter food web in a temperate deciduous forest, with emphasis on interactions between a community of generalist predators, the forest-floor spiders, and arthropod prey.

Elucidating occurrence of trophic interactions in the forest-floor food web was a formidable task due to the high diversity, small body sizes and cryptic habits of many litter-dwelling arthropods. Analysis of natural variation in consumer stable isotope ratios (δ¹³C and δ¹⁵N) formed the crux of this research because it simultaneously permitted quantification of the trophic positions of litter-dwelling arthropods and identification of spider resources, including prey subsidies from the grazing web. A monoclonal antibody-based ELISA was employed to analyze the gut contents of spiders to quantify predation on a major arthropod taxon, the forest-floor flies. Surveys of spider distributions and prey availability in the litter layer also provided fundamental knowledge of community structure.

Stable isotope analyses suggested that most spiders exhibited strong trophic connections to the detrital web, but weak links to herbivorous prey. Several lines of evidence supported a strong trophic link between large, litter-dwelling collembolans (Tomoceridae) and cursorial spiders, including correlation between spider and tomocerid densities on the forest-floor, similarities in spider and tomocerid carbon signatures, and nitrogen enrichment of tomocerids relative to other prey types. Conversely, this research provided conflicting evidence regarding spider consumption of flies. Gut content assays indicated consistent predation on flies by cursorial spiders, while stable isotope models suggested
that flies are likely of little importance in the spiders’ diets. This project yielded valuable insights into the role of spiders in the forest-floor food web and the potential importance of species-specific variation in prey consumption for detrital food web dynamics.

KEYWORDS: stable isotope analysis, gut content analysis, monoclonal antibody-based ELISA, Diptera, Collembola

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DISSERTATION

Erin Elizabeth Hladilek

The Graduate School
University of Kentucky
2009
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DISSERTATION

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CHAPTER I

Introduction

RESEARCH SUMMARY

Food webs present ecologists with a convenient means of describing complex relationships among organisms in nature. Food web structure, determined by the identities and behaviors of organisms comprising a community and patterns of connectance among those organisms, drives both community and ecosystem dynamics. Ecological investigations of terrestrial food web structure have traditionally focused on the aboveground components of the grazing food web, which is based on living plants. The key questions addressed by this research have primarily revolved around assessing the direct effects of grazing food web structure on primary productivity. Research on trophic interactions among litter and soil-dwelling fauna has been much less prolific despite growing interest amongst ecologists in belowground, detritus-based food webs in recent years.

However, the majority of primary productivity in terrestrial ecosystems is not consumed by grazing organisms, but rather is input directly into the detrital system. Detrital food webs thus control ecosystem function by regulating rates of decomposition, mineralization and nutrient cycling. As scientists are becoming increasingly concerned about the effects of global climate change, deforestation and changing land-use practices on ecosystem function, the study of detrital food web structure is currently a central theme in community and ecosystem ecology.

The research presented in this dissertation is focused on elucidating the structure of the detrital food web of an eastern, temperate deciduous forest, emphasizing interactions between the diverse community of litter-dwelling spiders and a complex assemblage of arthropod prey. I used a combination of complementary techniques to describe different aspects of the forest-floor food web including 1) field surveys to quantify predator community composition and examine species distribution within the habitat, 2) gut content analyses to examine spider predation on flies, and 3) analysis of natural variation in arthropod stable isotope ratios ($\delta^{13}$C & $\delta^{15}$N) to quantify detrital food web structure and resource utilization by forest-floor spiders. The following is a brief synopsis of the research conducted.

**Spider community composition microhabitat associations**

Detrital food web research often suffers from a lack of information regarding the identity and diversity of species occupying the litter or soil layers as well as the variance in spatial and temporal distribution of organisms within the habitat being studied. The objectives of this project were to 1) quantify forest-floor spider and prey community composition and 2) to assess spatial and temporal variation in horizontal distribution of spiders and prey in the litter layer in relation to a predominant forest-floor habitat characteristic, understory vegetation structure. A study was designed to test the hypothesis that small-scale variation in the distribution of predator and prey communities can result in spatial variability in trophic organization within the forest-floor habitat. Specifically, I examined the
correlations between understory vegetation density and prey availability and the density and diversity of litter-dwelling spiders.

The study revealed that densities of both cursorial and web-building spiders were significantly higher in microhabitats characterized by dense understory vegetation than in areas where understory vegetation was sparse. There was no concurrent correlation between the density of major arthropod prey groups and understory vegetation. There was however, a significant positive correlation between spider density and the abundance of one prey type, Tomoceridae (Collembola), which was significant only in areas with dense understory vegetation. The results of this study suggest that the spider community is not homogeneously distributed in the forest-floor habitat, but that patchily-distributed understory vegetation and other factors may influence trophic organization at the within-habitat scale. This study also supports previous research which suggests that tomocerid collembofans may be a particularly important resource for cursorial forest-floor spiders.

Species-specific variation in prey consumption by spiders
The small size and cryptic nature of many invertebrate organisms inhabiting the soil and leaf litter have led many food web ecologists to consider the detrital subweb as an ecological ‘black box.’ Lack of information about species diversity and dietary habits often results in creation of functional groups based on taxonomy, trophic relationships or habitat which can not only obscure important pathways of energy flow, but may lead to erroneous conclusions about the role of fauna in food web dynamics. Dietary information can be difficult to obtain for soil and litter fauna, as direct observations are rarely possible, microscopic techniques for gut content analyses are unable to detect ingestion of soft or liquefied food, and manipulating predator and prey populations in field experiments is often difficult. The objectives of this project were to assess consumption of a major group of arthropods, forest-floor flies, by two related spider taxa, the wolf spider *Schizocosa* spp. (Araneae, Lycosidae) and the wandering spider *Anahita punctulata* (Hentz) (Araneae, Ctenidae). The study employed a monoclonal antibody-based immunoassay to quantify spider consumption of flies via gut content analyses. Specifically, I tested the hypothesis that variation in resource utilization occurs between these spiders which can result in both species- and stage-specific functional roles for two very similar generalist predator species in the detrital food web.

The gut content survey revealed that all stages of both spider species consumed flies regularly throughout the spring, summer and fall, with peaks in consumption roughly correlating with peak flight activity of common forest-floor flies. There was no variation observed in the frequency of fly consumption between juveniles of the two species, or between juvenile spiders and adult (male and female) *Schizocosa* spp. However, female *A. punctulata* consumed flies significantly more frequently than did other juveniles or *Schizocosa* spp. These results suggest that juvenile spiders, which overlap in habitat utilization, body size and life history characteristics, utilize one major resource group in a similar manner. Conversely, adult *A. punctulata* seem to exhibit a shift in
utilization of flies which may result in functional separation of this species from *Schizocosa* spp. in the adult stage. The ontogenetic shift in prey selection may also effectively separate adults of this species from juveniles and decreased overlap in resource use may result in decreased competition within the species.

Leaf-litter food web structure and 'aboveground-belowground' links

Detrital food webs are typically thought to be characterized by highly reticulate trophic interactions, high species diversity and frequent occurrence of omnivory which results in lack of defined trophic levels. However, it has proven difficult to obtain empirical data on detrital food web structure because of the logistical constraints associated with observations of predation events. This component of the dissertation focuses on using stable isotope analysis ($\delta^{15}$N and $\delta^{13}$C) to examine detrital food web structure and predator-mediated linkages between the detrital and aboveground grazing webs. The objectives of this study were 1) to use natural variation in consumer nitrogen signatures to quantify forest-floor food web structure, focusing on trophic organization of the spider community, and 2) to use carbon source tracing to assess predator-mediated trophic connections between the grazing and detrital food webs on the forest-floor. I tested the hypotheses that 1) the leaf-litter food web is characterized by extensive omnivory among all organisms including generalist predators (i.e. intraguild predation) and 2) litter-dwelling spiders are highly polyphagous predators which function to link the detrital and grazing energy channels via consumption of herbivorous insect prey. I also tested the methodological hypothesis that significant differences in primary consumer (herbivore and microbe-detritivore) $\delta^{13}$C values, resulting from carbon fractionation during decomposition of plant matter, should allow for isotopic separation of the detrital and grazing food webs.

The results of this study suggested that the forest-floor detrital food web was characterized by frequent omnivory. However, omnivory resulted in three isotopically distinct ‘trophic levels’ related to resource utilization: specialist consumers of fungal hyphae, generalist consumers of detritus and associated microbes and generalist predators. Within the predator guild, variation in nitrogen signatures either indicated the frequent occurrence of intraguild predation, especially among cursorial species, or consumption of other $^{15}$N-enriched prey. One prey taxon, the flies, did not fit into the trophic-level structure based on resource utilization, but rather were highly enriched in $^{15}$N relative to all other organisms including predators. Flies $^{15}$N-enrichment was likely correlated with the degree of resource decomposition.

Significant differences $\delta^{13}$C values were observed between herbivorous insects and detritivorous or fungivorous arthropods allowing for tracing of the source of carbon assimilated by consumers. Therefore, a single-isotope mixing model was used to assess the contribution of resources from the detrital and grazing subwebs to the diets of litter-dwelling spiders. The model suggested that litter-dwelling spiders depend primarily on resources derived from the detrital food web. Estimates of spider biomass were used to assess the strength of trophic linkages between spider species and the detrital and grazing webs. The dominant spiders were cursorial species that did not appear to rely extensively
on prey subsidies from the grazing food web. Conversely, web-building species consumed a greater proportion of herbivorous prey, but represented a significantly smaller component of total spider biomass. Significant contributions of herbivorous prey were only evident in the diets of a few taxa, including a jumping spider (Salticidae) and a sac spider (Clubionidae), which forage actively in the vegetation. These results suggest that trophic connections between the detrital and grazing webs at the level of generalist predators are relatively weak, and these webs can be considered as relatively independent entities in the study ecosystem.

Modeling resource utilization by forest-floor spiders
Quantification of the contribution of diverse prey resources to diets of polyphagous predators in their natural habitats can be a particularly challenging aspect of food web research. The objectives of this study were to use stable isotope analysis to model contributions of several groups of potential arthropod prey to the diets of the wolf spider *Schizocosa* spp. and the wandering spider *Anahita punctulata*, and to compare the results of this model to expected patterns of resource utilization derived from the theory of ecological stoichiometry. Specifically I tested the hypothesis that spider dietary composition should be related to the value of available prey in terms of maintenance of homeostatic spider carbon-nitrogen ratios. Dependence on prey which fulfills requirements for a particular resource, such as nitrogen, could be indicative of selective consumption of prey by generalist predators in nature.

The isotope model utilized prey data gathered for the analysis of food web structure presented in Chapter IV. Prey isotope signatures were combined *a priori* using a clustering method to form seven statistically distinct prey groups, or clusters, which were used as endpoints in a dual-isotope, multi-source mixing model, IsoSource. The IsoSource model was used to calculate proportional contributions of each prey source to the diets of adults and juveniles of both species. The results of the model suggested that Tomoceridae (Collembola) were a key prey group for both spider species, and may be particularly important to juvenile stages. Flies generally contributed less to the diets of all spiders than did other prey types including macroarthropods, other collembolans and intraguild prey. However, ontogenetic shifts in spider δ^{15}N values indicated a switch to more ^{15}N-enriched prey (e.g. flies or intraguild prey) by adult spiders.

The mixing model analysis agreed strongly with predictions of prey consumption derived from stoichiometric theory. Analysis of spider and prey C:N ratios revealed that tomocerid collembolans contained a higher percentage of body nitrogen than did any other prey species collected, including other predators. Most prey taxa, including Entomobryidae (Collembola), flies, cockroaches (Dictyoptera), crickets (Orthoptera, Gryllidae) and moth larvae (Lepidoptera) were depleted in total nitrogen relative to predators and tomocerids. Millipedes (Diplopoda, Julidae) had extremely high C:N ratios and low nitrogen content relative to other arthropods. Stoichiometric constraints suggest that spiders feeding on most available prey may therefore experience nitrogen-limited growth and some groups, such as millipedes, are very poor
quality prey. Tomocerids would conversely provide spiders with an excess of nitrogen, while cannibalism or intraguild predation would provide sufficient nitrogen to allow spiders to avoid nitrogen limitation. This extreme disparity in prey value and contribution of valuable prey groups to the diets of spiders suggests that prey selection by generalist predators in the detrital food web is not likely to be random or correlated only with the availability of prey in the environment.

STUDY SITE DESCRIPTION
All research was conducted in the Berea College Forest, a second-growth mixed-hardwood forest located in Madison County, Kentucky, USA (37°34’ N, 84°13’ W). The forest canopy was dominated by oaks (Quercus spp.), hickories (Carya spp.) and red maples (Acer rubrum L.) with scattered pines (Pinus spp.). The understory was composed of tree seedlings and saplings, various shrubs including sassafras (Sassafras albidum (Nutt.)), mountain laurel (Kalmia latifolia L.) and blueberries (Vaccinium spp.), and vines (Smilax spp.). Herbaceous vegetation was relatively uncommon on the forest floor with the exception of poison ivy (Toxicodendron radicans (L.)) which was widely distributed in gaps, along trails and near stream beds. The soil at the site was a highly acidic silt loam (USDA web soil survey 2007) covered by a relatively thin organic horizon (2.5 – 10 cm). Average annual rainfall in this area totals approximately 120 cm, mean temperatures range from -4°C to 30°C and the growing season is approximately 190 days (climate data courtesy of the UK Agricultural Weather Center, Climatology, collected at Berea, KY).
CHAPTER II

Leaf-litter spider community composition and microhabitat associations

SUMMARY

Environmental heterogeneity can influence arthropod community composition at scales ranging from an entire ecosystem to a single plant. Much food web research has either been based on the assumption that food web structure within a habitat is static, or that small-scale variation does not significantly influence the food web as a whole. Small-scale spatial variation in community structure has been frequently well-studied in the context of detrital food webs, which are characterized by vertical stratification in the soil and litter microhabitats. Horizontal distribution of litter fauna, especially highly mobile generalist predators, has been less frequently quantified. This study examined within-habitat distribution of litter-dwelling spiders and prey in relation to understory plants in a temperate deciduous forest. Open plots were established in areas with either dense or sparse understory vegetation cover. Spiders and potential arthropod prey were sampled on three occasions during the growing season, early, mid- and late summer, using a combination of pitfall traps and litter samples. The survey revealed that both cursorial and web-building spiders were more abundant in areas with dense vegetation, particularly at the mid- and late-season sampling intervals. This pattern was driven primarily by the distributions of juveniles of three numerically abundant spider taxa, *Phrurotimpus* spp. (Araneae, Corinnidae), *Anahita punctulata* (Araneae, Ctenidae) and *Xysticus* spp. (Araneae, Thomisidae). Collembolans, the dominant group of arthropod prey collected, were not correlated with understory vegetation density. However, the density of cursorial spider species was observed to be positively correlated with density of one prey taxon, the Tomoceridae (Collembola), and this association was significant only in areas with dense understory vegetation. This supports the hypothesis that within-habitat spatial heterogeneity may affect local predator-prey interactions on the forest floor. Possible mechanisms explaining the observed patterns in spider and prey distributions, potential implications for overall food web structure and directions for future research are discussed.
INTRODUCTION

Studies documenting the structure of complex food webs traditionally focus on habitat- or ecosystem-level variation in community composition and effects on trophic relationships (e.g. Polis et al. 1997). However, environmental heterogeneity is a defining feature of many systems which can drive small-scale variation in trophic organization and can facilitate biodiversity and alter ecosystem function. The importance of spatial and temporal variability in species composition to detrital food web dynamics has been explored primarily in the context of vertical stratification of fauna in the mineral soil and organic horizons (Berg et al. 1998, Wagner et al. 2003, Berg and Bengtsson 2007). Horizontal variation in soil and litter food webs has been less studied, though small-scale patterns in the distribution of soil biota related to plant community composition or other environmental variables may play a significant role in detrital food web dynamics (Ettema and Wardle 2002). In particular, the distribution of predators within heterogeneous habitats can have a significant impact on local prey populations. Selective use of optimal microhabitats, or predator movement among patchily-distributed habitats, may both act to stabilize predator community dynamics and allow prey species to escape from predation (Brose et al. 2005). The current study examines the composition of the spider-dominated generalist predator community of the complex detrital food web of a deciduous forest, and the distribution of spider species in relation to microhabitat heterogeneity and prey availability.

In the temperate deciduous forest, environmental conditions on the forest floor are regulated by spatial and temporal patterns in plant community structure. Plant identity and diversity can affect litter quantity and quality, which can affect detrital food web structure (reviewed by Wardle 2002). However, physical characteristics of the aboveground vegetation, such as understory plant density or architectural complexity, may also directly impact the suitability of the forest-floor habitat for arthropod fauna, such as spiders (e.g. Bultman and DeWitt 2008). Previous research of the effects of microhabitat complexity on ground-dwelling spiders has focused on understanding the effects of litter structure on spider population and community dynamics (reviewed by Uetz 1991). These studies have shown that both cursorial spider species (e.g. species which do not rely on webs for prey capture) and web-building spiders are positively influenced by litter complexity in the forest-floor habitat (Uetz 1979). The mechanisms driving spider population or community responses to litter structure are varied, but may include high prey density or diversity associated with greater litter volume, increased three-dimensional structure which supports species that forage in the litter layers and use curled leaves as retreats (e.g. Gnaphosidae, Corinnidae, Thomisidae) or more favorable microclimate conditions associated with deeper litter (Uetz 1975, 1976, 1979). Spiders are particularly sensitive to small-scale variations in climatic conditions due to the low tolerance to desiccation and extreme temperatures (Pulz 1987), so increased moisture availability and decreased temperature fluctuations associated with a deep
litter layer may favor higher densities of spiders. Web-building spider density and diversity are also positively correlated with the structural complexity of the leaf litter layer because rigid, curled leaves with high interstitial volume can provide more sites for web attachment than can thin or compressed leaf litter (Turnbull 1973, Stevenson and Dindal 1982, Bultman and Uetz 1982, 1984).

Although most research on microhabitat selection by forest-floor spiders has considered leaf litter characteristics, vegetation structure may also have a significant effect on spider population dynamics and community composition. Previous research at the habitat scale has demonstrated that forest-floor spider density and diversity is correlated with tree species identity, probably as a result of the quantity, quality or structure of the litter layer (Pearce et al. 2004, Schuld et al. 2008, Ziesche and Roth 2008). Understory, and particularly ground-level vegetation, may directly affect the distribution of web-building spiders by providing architectural support for web attachment. Positive correlations between the availability of web attachment points and the density of foliage-dwelling orbweavers have been observed in agricultural and forest ecosystems (Robinson 1981, Balfour & Rypstra 1998, McNett and Rypstra 2000, Miyashita and Takada 2007). Similarly, ground-dwelling web-builders may utilize the rigid support structures offered by plant stems to anchor webs at or near the litter surface. Cursorial spiders are not likely to be dependent on any particular aspect of vegetation architecture, though canopy and ground-level vegetation structure may have a significant impact on the spider community by influencing microclimatic conditions on the litter surface (Oxbrough et al. 2005, Zeische and Roth 2008). Vegetation structure may also mediate predator-prey interactions in the detrital food web by altering prey encounter rates or a predator’s ability to detect and capture prey. For example, structurally complex or deep litter may impede a rapid escape response by prey, which is the primary defense against predation utilized by most litter-dwelling Collembola (Hopkin 1997). Ground-level vegetation may have similar effects on forest-floor predator-prey interactions, though this has not been investigated. The objectives of this study were therefore to quantify spatial and temporal variation in spider species distribution in the forest-floor habitat in relation to heterogeneous understory vegetation. I tested the hypothesis that local spider density and community composition is correlated with understory microhabitat structure. Specifically, spiders should be more abundant and diverse in the complex microhabitat provided by patchily-distributed, dense understory vegetation. Further I assessed prey availability and correlations between spider and prey densities across the range of available microhabitats in order to discern patterns of variation in trophic organization within the forest-floor habitat.
MATERIALS AND METHODS

Sampling methods
Three 100 x 100m (1-ha) blocks, separated by approximately ½ km, were selected for the arthropod survey. Within each block, five open experimental plots (8 x 8m) were established in areas characterized by dense understory vegetation, and five plots were placed in areas with sparse understory vegetation (Figure 2.1). The criterion used to select densely-vegetated areas was that more than 50% of the litter layer was visually obscured by foliage from shrubs, seedlings, vines and herbs (< 1 m high). The criterion for identifying sparsely-vegetated areas was that bare litter was visible over at least 90% of the forest floor. The estimates of foliage cover were made visually, by a single observer standing at the edge of the plot. Since a finite number of areas fitting the desired habitat criteria were available within the confines of each block, a pseudo-random process was employed to select the areas included in the study. Initially ten areas representing each of the two habitat types were located in each block and mapped using a handheld differentially-corrected global positioning system (GPS) unit. The plot maps were examined for interspersion of the habitat types and dispersal of plots within the site. Five plots of each habitat type were selected which maximized interspersion and maintained a minimum distance of 10m between adjacent plots. This selection method was necessitated due to non-random dispersal of densely vegetated plots around gaps in the canopy resulting from one or multiple tree falls.

Measurements of arthropod population and community parameters were made using two complementary sampling methods, litter extraction and pitfall trapping. Quantitative estimates of spider and prey densities were obtained by hand-searching samples of leaf litter collected from the forest floor. Two samples of litter, each 0.25 m$^2$, were individually shaken through a large (1.5 m) mesh sifter to remove large litter fragments and dislodge organisms. Invertebrates were sorted from the fine debris which was collected in a large tub, and placed in 70% EtOH for later identification. All litter samples were searched twice by a single experienced collector, to ensure repeatability and the most complete extraction of arthropods. Following extraction, the litter was carefully returned to its original location in the plot, though sifted areas of the forest floor were never searched more than once during the course of the summer. Each set of litter samples was collected during the course of 3-4 consecutive days unless delayed by rain. Arthropod activity-density was monitored using pitfall traps. Four traps were established in each plot in the spring at least two weeks prior to the first sampling period. The traps were constructed from two disposable plastic drinking cups (7 cm diameter) buried so that the lip of the inside cup was flush with the surface of the soil. The inner cup was removable and contained approximately 100 mL 70% EtOH as a preservative. A plastic funnel was inserted into the top of each cup to deter escape by climbing species. Traps were opened for 96 hrs at each sampling date, and closed when not in use to avoid local depletions in
population densities which can result from continuous long-term trapping. Pitfall traps and litter samples were collected on three occasions during 2006, representing early, mid and late summer activity periods. The early summer samples ended on May 30 and June 15 for litter and pitfall samples respectively, July 11 and August 3 for the mid-summer samples, and August 20 and September 15 for the late summer samples. Spiders collected were identified to genera using Ubick et al. (2005) and to species using appropriate keys whenever possible. All potential prey organisms were identified to the family level.

**Statistical analysis**
The effects of vegetation density and season on spider and prey density and activity-density were analyzed using separate multivariate analyses of variance (MANOVA), with habitat and season as fixed effects. Spider and prey families were used as dependent variables in the MANOVA. Univariate ANOVA’s were used to compare habitat associations for individual taxa if the MANOVA results showed a significant habitat effect for spider families. Correlations between spider and prey populations were modeled using a backwards stepwise multiple regression. Data were transformed using either log or square root transformations to approximate normality and achieve homogeneity of variances if necessary prior to analysis. Statistical analyses were performed using SAS 8.01 (SAS Institute, Cary, NC).

**RESULTS**

**Spider community composition**
A total of 958 spiders from 13 families were collected from the leaf litter samples (Table 2.1). Cursorial species represented 63% of ground-dwelling spiders captured using this method, while web-builders were 37%. The dominant cursorial species were running spiders in the families Corinnidae (21%) and Gnaphosidae (16%); sit-and-wait predators, Lycosidae (12%) and Ctenidae (6%); stalking predators, Salticidae (5%); and ambush predators, Thomisidae (4%). The dominant web-building spiders were tiny sheetweb weavers in the families Linyphiidae (14%) and Dictynidae (8%) and vagrant web-builders, Titanocidae (7%) and Segestriidae (7%). Pitfall trapping resulted in the capture of 2221 litter-dwelling spiders from 19 families (Table 2.1). However, pitfall traps are ineffective for sampling many web-building spiders or species which can escape from the traps (Wagner et al. 2003). This is emphasized by the fact that more than 91% of the individuals identified from pitfall captures were cursorial spiders, with Lycosidae (56%) and Gnaphosidae (8%), Salticidae (8%) and Corinnidae (7%) representing the majority of spiders captured. *Schizocosa* spp. (*S. stridulans*, *S. saltatrix* and *S. ocreata*) were the dominant wolf spiders at the study site, and represented more than 79% of all lycosids captured, while a second abundant wolf spider, *Arctosa virgo*, represented nearly 20% of individuals. More than 60% of wolf
spiders collected by pitfall traps were cursorial males captured during the spring breeding period, while adult females represented a much smaller proportion of total individuals captured (14%). Some cursorial spiders which were abundant in the leaf-litter, including Corinnidae (7%) and Ctenidae (3%). were poorly represented in pitfall trap captures, either due to inactivity or the ability to avoid or escape from the traps. High numbers of web-building Dictynidae (3%) captured in the pitfall traps were predominantly wandering male *Cicurina* spp. that were actively searching for mates at the time of collection.

**Microhabitat associations**

Results of the MANOVA indicated that there was a significant effect of understory habitat type on the densities of both cursorial and web-building spiders (Table 2.2). Spider density was significantly higher in areas characterized by dense understory than in the surrounding sparsely vegetated areas (Figure 2.2). Univariate analyses indicated that the correlation between understory vegetation and spider density differed between families. Among the cursorial spiders, significant effects of habitat type were found for Corinnidae (*F* = 5.58, *p* = 0.02), Ctenidae (*F* = 11.34, *p* = 0.001) and Thomisidae (*F* = 11.04, *p* = 0.02), but not for Lycosidae or Gnaphosidae (Table 2.3). *Anahita punctulata* (Figure 2.3) and *Phrurotimpus* and *Xysticus* spp. (Figure 2.4) were more abundant in the densely vegetated plots than in areas with a sparse understory. Among web-building families, habitat effects were seen in tiny sheetweb and meshweavers, Linyphiidae (*F* = 7.14, *p* = 0.02) and Dictynidae (*F* = 5.73 *p* = 0.01), but not for the larger web-builders in the families Segestriidae or Titanocidae (Table 2.3). As was the case for cursorial spiders, the small web-builders were significantly more abundant in the dense understory vegetation (Figure 2.4).

Conversely, the MANOVA showed that there was no correlation between the occurrence of understory vegetation and the activity-density of either cursorial (*F* = 1.95, *p* = 0.08) or web-building spiders (*F* = 1.24, *p* = 0.29) as measured by pitfall traps. This analysis, however, excluded *Schizocosa* spiderlings, which were captured in large numbers in pitfall traps, comprising approximately 20% of total *Schizocosa* individuals captured. Spiderlings were excluded from the activity-density analysis because female wolf spiders carry spiderlings on their abdomens for a period of several days following hatching. Thus, first instar spiderlings were presumed to have fallen into the traps en masse while being carried on the abdomen of an adult female *Schizocosa*, and one or more females were always found in traps containing large numbers of spiderlings. In this case the actual number of spiderlings entering the trap is likely related to developmental stage and fecundity of the female, and not to activity of the spiderlings being counted. However, the presence or absence of large numbers of spiderlings in a plot is indicative of the behavior of spiderling-carrying females, which may differ from that of other females. In this case there was a significant difference between spiderling
occurrence and understory vegetation, with nearly all first-instar *Schizocosa*
spiderlings collected in sparsely-vegetated areas. Eight of fifteen sparse plots
sampled in July had one or more traps containing large numbers of
*Schizocosa* spiderlings (10 – 35 individuals), while there were no traps
containing large numbers of spiderlings in densely vegetated areas, indicating
preference for open leaf litter in brood-carrying female wolf spiders.

In addition to habitat effects, there was significant temporal variation in
cursorial spider density and activity-density ($F_{12,156} = 51.08$, $p < 0.0001$).
Cursorial spider density was significantly higher in the late-season sample,
while web-builder density peaked in mid-summer (Figure 2.2). Conversely,
cursorial spider activity-density peaked in the early summer sample, coincident
with the presence of high numbers of male wolf spiders searching for mates,
and decreased through the season. Web-builder activity-density was
consistently low throughout the summer (Figure 2.2).

**Prey availability**
Collembola were the numerically dominant detrital prey taxon collected in both
leaf litter and pitfall samples from the forest floor. Five collembolan families
were collected; Tomoceridae, Entomobryidae, Sminthuridae, Isotomidae and
Hypogastruridae. Tomoceridae were the dominant taxa, representing 91% of
all collembolans collected from the litter samples and 53% of individuals in the
pitfall traps (Figure 2.6). Entomobryidae were 24% of the total collembolan
capture in the pitfall traps, but represented only 6% of collembolans from the
litter samples. Sminthuridae and Isotomidae were likewise rarely collected
using the litter sifting method, but represented 13% and 5% of the pitfall trap
capture, respectively. Hypogastrurids were occasionally captured in large
numbers in pitfall trap samples (> 500 individuals per trap) but were not
consistently present in traps or litter samples. Additionally, some
hypogastrurids produce chemical feeding deterrents which may make them
unpalatable to most spiders (Bitzer et al. 2004), so they will not be considered
further in this analysis. Other detritivores and omnivores commonly collected
were crickets (*Gryllus* sp. and *Acheta domesticus*), wood roaches (*Parcoblatta*
spp.), millipedes, flies and Lepidoptera larvae. Two other dominant detrital
taxa which may be important components of this food web, oribatid mites
(Acari, Oribatida) and flies (Diptera) were not adequately measured by the
sampling techniques used in this study (Chen and Wise 1999).

A MANOVA which incorporated all abundant prey groups showed that
there was no significant effect of habitat type on detritivore density in the litter
(Wilk’s $\lambda = 0.9960$, $F_{3,82} = 0.11$, $p = 0.95$). Detritivore activity-density was also
unrelated to habitat type (Wilk’s $\lambda = 0.8705$, $F_{7,77} = 1.64$, $p = 0.14$). There was
a significant effect of season on detritivore density (Wilk’s $\lambda = 0.7543$, $F_{6,164} =
4.14$, $p = 0.0001$) as well as on activity-density (Wilk’s $\lambda = 0.0791$, $F_{14,154} =
28.10$, $p < 0.0001$). There was no significant correlation observed between
spider and prey activity-densities measured by pitfall trapping. However,
cursorial spider density was positively correlated with density of a single prey
group, tomocerid Collembola, and this correlation was affected by habitat type. While there was only a very weak, but significant positive correlation between predator and prey numbers in the sparsely vegetated habitats ($y = 1.25x + 4.81$, $r^2 = 0.09$, $p = 0.04$), there was a moderately strong correlation between spiders and tomocerids in densely vegetated plots ($y = 3.87x - 2.33$, $r^2 = 0.45$, $p < 0.0001$) (Figure 2.7a). While cursorial spiders were more abundant in the densely vegetated plots, there was no difference in tomocerid density between the two habitat types. There was no similar correlation between web-building spiders and tomocerids in either dense ($p=0.25$) or sparse vegetation ($p=0.38$) (Figure 2.7b).

**DISCUSSION**

Both cursorial and web-building spiders were more abundant in microhabitats characterized by dense understory vegetation than in areas with sparse vegetation. In the case of web-building spiders, the most parsimonious explanation of this pattern is that architectural features associated with dense or diverse patches of plants, such as high density of stems, roots or low-lying foliage, provide greater availability of web attachment points. Previous research has shown that vegetation complexity is positively correlated with the density of sheetweb weavers (Linyphiidae) in agroecosystems (Alderweireldt 1994, Balfour and Rypstra 1998). Sheetweb weavers (Erigoninae and Linyphiinae) and the meshweaver *Lathys immaculata* (Chamberlin & Ivie) (Dictynidae) were the numerically dominant representatives of the web-building guild in the forest-floor food web. Both groups are predominantly found in the middle and lower litter layers (Wagner et al. 2003) where they may use the basal structure of dense seedlings or other plants to support webs. These taxa were only more abundant under dense vegetation at the mid-summer collection period when densities of both groups were relatively high, which suggests that websites are a limiting factor in areas of sparse vegetation. The other common web-builders inhabiting the forest floor, *Ariadna bicolor* (Segestriidae) and *Titanoerca americana* (Titanoecidae) showed trends towards higher densities in sparsely vegetated areas, though this was not statistically significant. *Titanoerca americana* likely constructs small retreat webs in the litter (Leech 1972), while *A. bicolor* inhabits tubular webs built under loose bark, stones or litter, which do not require vertical support (Ubick 2005). For the most part none of the web-builders in the litter layer were particularly active off of their webs, as evidenced by the absence of large numbers of these species in the pitfall trap collections.

The mechanisms driving the association between cursorial spider species and dense understory vegetation are probably more complex. The observed pattern resulted from responses by three numerically dominant spider taxa, *Phrurotimpus* spp. (*P. alarius* (Hentz) and *P. borealis* (Emerton)) (Corinnidae), *Anahita punctulata* (Hentz) (Ctenidae) and *Xysticus* spp. (Thomisidae), to vegetation density. *Phrurotimpus* spp. were numerically dominant in the litter layer and exhibited the strongest positive association with
dense understory vegetation in both absolute density and activity. *Phrurotimpus alarius*, the dominant species at the study site, is a spring-breeding species with adults present in May and June, and juveniles increasing in abundance throughout the rest of the summer and fall (Draney 1997). There was no evidence of preferential microhabitat utilization by this species during the first, late-spring sampling period when most individuals collected were adults. However, *Phrurotimpus* spp. activity-density and abundance increased in the densely vegetated plots throughout the summer as juveniles were produced, indicating an ontogenetic shift in habitat utilization. The tiny size of spiders (<3 mm) makes them potential prey for larger litter-dwelling spider species, such as wolf spiders, and complex vegetation structure may decrease juvenile mortality rates resulting from predation (e.g. Finke and Denno 2002, Rickers et al. 2006). Similar patterns were evident for *A. punctulata* and *Xysticus* spp., which were similarly distributed in sparsely and densely vegetated microhabitats early in the season when most adults were present. In both cases, juvenile spider density increased in the dense microhabitat throughout the summer and fall, but stayed low in the sparsely vegetated microhabitat.

Other dominant cursorial spiders in the forest-floor food web, including the wolf spiders *Schizocosa* spp. and *Arctosa virgo* (Chamberlin) (Lycosidae), the stealthy ground spiders, *Gnaphosa fontinalis* Keyserling and *Drassyllus* spp. (Gnaphosidae), and the jumping spider *Phidippus whitmani* Peckham & Peckham (Salticidae), did not exhibit any preference for understory vegetation density. Wolf spiders are generally considered to be sit-and-pursue predators (Uetz et al. 1999), that locate prey using visual and vibratory cues, wait until the prey comes within range and then actively pursue the prey prior to subduing it (Rovner 1980). In this case dense vegetation may actually impede the spiders’ ability to detect or subdue prey. Conversely, young wolf spiders may be subject to limitation by IGP or cannibalism (Wagner and Wise 1996, Chen and Wise 1999), and dense vegetation may allow spiders to hide from predators, or climb to safety (Folz et al. 2006), which could balance potential negative effects of vegetation density on prey capture (e.g. Rypstra et al. 2007). It is also possible that other habitat characteristics, such as litter depth, structure, moisture availability are simply more important than aboveground vegetation for many litter-dwelling spider species.

The microhabitat survey suggested that the density of understory vegetation has no effect on the spatial distribution of one dominant group of forest-floor microbi-detritivores, the Collembola, which may serve as an important resource for litter-dwelling spiders in this system (Chen and Wise 1999, Lawrence and Wise 2000, 2004, Wise 2004). This is not surprising, as the spatial distribution and density of many collembolan species is thought to be controlled by soil or litter moisture availability in conjunction with production of social aggregation pheromones rather than by other habitat characteristics (e.g. Grear and Schmitz 2005, Vegter et al. 1988, Verhoef and Nagelkerke 1977, Verhoef et al. 1977). However, previous research has demonstrated
that large litter-dwelling collembolans do exhibit spatial and temporal aggregations in the forest-floor habitat (Grear and Schmitz 2005). The current analysis, which integrated spatial and temporal patterns in arthropod distributions, revealed a positive correlation between the density of cursorial spiders and the abundance of the dominant collembolan family, Tomoceridae, in the leaf litter. There are two possible explanations for this phenomenon. The first is that spiders and Tomoceridae are responding to similarly favorable microhabitat conditions, specifically moisture availability. If dry conditions are prevalent, drought-sensitive tomocerids may be restricted to wet areas (Verhoef and Nagelkerke 1977) which would likewise be favorable to spiders that are intolerant of desiccation (Pulz 1987). This mechanism seems unlikely because there were no differences in tomocerid density or activity in areas with dense versus sparse understory vegetation, yet the correlation between spider and tomocerid densities was only significant in areas characterized by dense understory vegetation. The second possible explanation for the correlation between spider and tomocerid densities is a numerical response by spiders to high prey densities. Aggregation to areas of high prey density may be a common foraging strategy for some mobile predators, such as ground beetles (Coleoptera, Carabidae) (e.g. Bryan and Wratten 1984, Winder et al. 2001), and should result in positive spatial correlations between predator and prey densities if the prey is relatively immobile (Sih 1984). However, such correlations have rarely been recorded for spiders in nature. In one exception, Harwood et al. (2001a, 2003) found positive correlations between the location of web sites of mobile dwarfweavers (Linyphiidae, Erigoninae) and the availability of Collembola in a relatively simple, homogeneous agricultural habitat. In the forest-floor habitat, the association between spiders and tomocerids was strongest in areas of dense understory vegetation where spider density was highest. Dense, complex vegetation structure may increase the efficiency of prey capture for spiders by impeding rapid escape behavior which forms the basis of the collembolan anti-predator defense strategy (Bauer 1982, Hopkin 1997).

The interaction between forest-floor spiders and Collembola has been the focus of previous research on detrital food web dynamics in this system. Chen and Wise (1999) found that increasing densities of Collembola and other fungivorous arthropods in the litter layer resulted in increased density and biomass of spiders. This suggests that spiders in the leaf-litter food web are subject to resource limitation and are dependent on the availability of detrital prey. Tomocerid Collembola have been specifically implicated as an important resource for spiders in this food web, as experimental decreases in cursorial spider density led to significant increases in tomocerid numbers in the litter layer as a result of reduced predation pressure (Lawrence and Wise 2004, Wise 2004). This result substantiates the current observations that spiders are closely associated with tomocerids in the forest-floor habitat, though the reasons for strong trophic interactions between cursorial spiders and this particular prey group are elusive. It is possible that the complex biotic and
abiotic variables which govern distributions of spiders and tomocerids promote substantial spatial and temporal overlap of these organisms in the litter habitat. For example, large collembolans, such as Tomoceridae, are most abundant and active in the upper and middle litter layers on the forest floor which coincides with the vertically-stratified distribution of cursorial spiders in the litter (Berg et al. 1998, Wagner et al. 2003). Another explanation for trophic links between cursorial spiders and tomocerids is the occurrence of active prey selection. While prey consumption by spiders and other generalist predators is often proportional to prey encounter rates in the environment, other factors which may influence resource utilization include prey quality, ease of capture and risk associated with predation. Laboratory research has demonstrated that spiders are able to assess nutritional quality of potential prey items and optimize consumption based on physiological requirements (Greenstone 1979, Mayntz et al. 2005), and Tomoceridae have been observed to be important high-quality prey for ground-dwelling spiders in laboratory studies (Toft and Wise 1999a).

This research has illustrated that complex interactions between microhabitat characteristics (e.g. vegetation structure) and predator-prey distributions in a heterogeneous environment may create within-habitat variation in food web structure. This further underscores the need to consider small-scale spatial and temporal variation in trophic organization when examining the structure of complex food webs. While this study highlighted interesting patterns in the distribution of forest-floor spiders and spatio-temporal associations between cursorial spiders and collembolans, experimental research is necessary to assess the mechanisms driving these patterns and the magnitude of their effect, if any, on detrital food web dynamics.
Table 2.1 Spider community composition in the forest-floor leaf litter

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Pitfall captures</th>
<th>Litter samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>%</td>
</tr>
<tr>
<td><strong>Cursorial spiders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Schizocosa</em> spp.</td>
<td>Lycosidae</td>
<td>990</td>
<td>43.9</td>
</tr>
<tr>
<td><em>Arctosa virgo</em> (Chamberlin)</td>
<td>Lycosidae</td>
<td>249</td>
<td>11.0</td>
</tr>
<tr>
<td><em>Phrurotimpus</em> spp.</td>
<td>Corinnidae</td>
<td>146</td>
<td>6.5</td>
</tr>
<tr>
<td><em>Phidippus whitmani</em> Peckham&amp;Peckham</td>
<td>Salticidae</td>
<td>121</td>
<td>5.4</td>
</tr>
<tr>
<td><em>Gnaphosa fontinalis</em> Keyserling</td>
<td>Gnaphosidae</td>
<td>104</td>
<td>4.6</td>
</tr>
<tr>
<td><em>Xysticus</em> spp.</td>
<td>Thomisidae</td>
<td>72</td>
<td>3.2</td>
</tr>
<tr>
<td><em>Anaita punctulata</em> (Hentz)</td>
<td>Ctenidae</td>
<td>56</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Salticidae</em> spp.</td>
<td>Salticidae</td>
<td>52</td>
<td>2.3</td>
</tr>
<tr>
<td><em>Drasyllus</em> spp.</td>
<td>Gnaphosidae</td>
<td>50</td>
<td>2.2</td>
</tr>
<tr>
<td><em>Antrodiaetus unicolor</em> Gertsch</td>
<td>Antrodiaetidae</td>
<td>25</td>
<td>1.1</td>
</tr>
<tr>
<td><em>Castianeira cingulata</em> (Koch)</td>
<td>Corinnidae</td>
<td>11</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td><em>Zelotes</em> spp.</td>
<td>Gnaphosidae</td>
<td>10</td>
<td>&lt;1.0</td>
</tr>
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<td><em>Hogna aspera</em> (Hentz)</td>
<td>Lycosidae</td>
<td>9</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td><em>Litopyllus temporarius</em> Chamberlin</td>
<td>Gnaphosidae</td>
<td>8</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td><em>Ummidia</em> sp.</td>
<td>Ctenizidae</td>
<td>7</td>
<td>&lt;1.0</td>
</tr>
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<td><em>Dolomedes</em> sp.</td>
<td>Pisauridae</td>
<td>5</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td><em>Elaver excepta</em> (Koch)</td>
<td>Clubionidae</td>
<td>3</td>
<td>&lt;1.0</td>
</tr>
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<td><em>Pirata</em> sp.</td>
<td>Lycosidae</td>
<td>2</td>
<td>&lt;1.0</td>
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<tr>
<td><em>Pisaurina mira</em> (Walckenaer)</td>
<td>Pisauridae</td>
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</tr>
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<td><em>Sphodros</em> sp.</td>
<td>Atypidae</td>
<td>2</td>
<td>&lt;1.0</td>
</tr>
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<td><em>Cesonia bilineata</em> (Hentz)</td>
<td>Gnaphosidae</td>
<td>1</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td><em>Rabdosa rabida</em> (Walckenaer)</td>
<td>Lycosidae</td>
<td>1</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td><em>Platycryptus undatus</em> (DeGeer)</td>
<td>Salticidae</td>
<td>1</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td><em>Ozyptila</em> sp.</td>
<td>Thomisidae</td>
<td>1</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td><strong>Web-building spiders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cicurina</em> spp.</td>
<td>Dictynidae</td>
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Table 2.2 MANOVA for effects of microhabitat and date on spider density

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Table 2.3 Univariate ANOVA’s for effects of microhabitat and date on spider densities

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a) Dense understory microhabitat

Figure 2.1 Variation in forest-floor microhabitat resulting from patchy distribution of the understory vegetation.

b) Sparse understory microhabitat
Figure 2.2 Comparison of microhabitat associations and seasonal trends in a) density and b) activity-density of cursorial and web-building spiders.
Figure 2.3 Comparison of microhabitat associations and seasonal trends in a) density and b) activity-density of sit-and-wait predators.
Figure 2.4 Comparison of microhabitat associations and seasonal trends in a) density and b) activity-density of active-hunting predators.
Figure 2.5 Comparison of microhabitat associations and seasonal trends in a) density and b) activity-density of web-building spiders.
Figure 2.6 Comparison of microhabitat associations and seasonal trends in a) density and b) activity-density of litter-dwelling Collembola.
Figure 2.7 Correlations between spider and Tomoceridae (Collembola) densities for a) wandering spiders and b) web-building spiders.
CHAPTER III

Gut content analysis reveals variation in prey consumption between syntopic forest-floor spiders

SUMMARY

Arthropod generalist predators, such as spiders, are often categorized into functional groups based taxonomic relationships or foraging habits, in studies of food web structure and dynamics. However, recent research suggests that species- and possibly stage-specific variation in prey consumption, foraging habits or predator behavior can influence prey populations and community dynamics. This study quantified predation on forest-floor flies (Diptera) by two species of large, litter-dwelling cursorial spiders, *Schizocosa* spp. (Araneae, Lycosidae) and *Anahita punctulata* (Hentz) (Araneae, Ctenidae). The frequency of fly consumption by spiders in the forest-floor habitat was determined by gut content analysis using a monoclonal antibody-based ELISA. The analyses revealed that both species regularly consumed flies throughout the summer, though peaks in consumption occurred in spring and fall concurrent with peaks in fly activity. There were no apparent differences in fly consumption between juveniles of the two species however, adult female *A. punctulata* consumed flies significantly more frequently than did juvenile spiders or adult *Schizocosa* spp. The lack of differences in prey utilization between juveniles of these spiders implies some level of trophic redundancy at least with regards to this particular prey group. The ontogenetic shift in fly consumption by adult *A. punctulata* suggests that these spiders may occupy a slightly different ecological role than do *Schizocosa* spp. and hence may not be completely redundant in their effects on forest-floor fly populations.
INTRODUCTION

Concerns about changes in natural ecosystems, particularly loss of biodiversity, have resulted in a plethora of ecological studies of the fundamental relationships between community composition and ecosystem function (reviewed by Tylianakis et al 2008). A general conclusion of this research is that variation in the functional characteristics of the species comprising a community can affect ecosystem-level dynamics (Hooper et al. 2005). This research highlights a significant shortcoming of many studies of food web structure; the practice of lumping species into functional groups on the basis of taxonomic relationship, trophic level or other factors (Polis 1991, reviewed by Hawkins and MacMahon 1989). The integrity of such functional groups is based on the assumption that all species in the group utilize resources in the same manner and have similar effects on population and community dynamics. However, experimental research suggests that even closely related species which appear to fulfill similar ecological roles (e.g. generalist predators) can have significantly different impacts on food web dynamics (Chalcraft and Resetarits 2003, O’Connor and Crowe 2005). The objective of this research was to quantify prey consumption by two syntopic spiders, wolf spiders, *Schizocosa* spp. (Araneae, Lycosidae) and the wandering spider, *Anahita punctulata* (Hentz) (Araneae, Ctenidae), inhabiting the leaf litter layers of a temperate deciduous forest, in order to determine whether significant species- or even stage-specific variation in predator-prey interactions might influence detrital food web dynamics.

The use of functional groups has been particularly pervasive in detrital food web research (e.g. Moore et al. 1988) due to the lack of dietary information (Polis 1991) for the small, cryptic polyphagous species which inhabit the soil and litter layers of most terrestrial ecosystems. In the case of spiders, relatively little quantitative evidence regarding prey consumption exists for non-agricultural species, because it is difficult to observe relatively infrequent predation events in nature (e.g Nyffeler and Benz 1981, 1988, Nyffeler and Breene 1990), especially when predation occurs in the soil or litter. Gut content analysis has frequently been used to assess trophic interactions between arthropod generalist predators, such as ground beetles (Coleoptera, Carabidae) and detritivorous or fungivorous arthropods (reviewed by Hladílek 2003). Until recently, however, these analyses have primarily relied upon microscopic identification of partially digested prey remains, and therefore require the presence of sclerotized, indigestible prey in the predators’ gut contents. Microscopic gut content analysis is therefore useless for quantifying prey consumption by spiders and other organisms which ingest food in a liquid state (reviewed by Sunderland 1987). The use of immunoassays for prey detection, especially enzyme-linked immunosorbent assay (ELISA), has largely resolved this problem and recent advances in the development and use of monoclonal antibodies have greatly improved our ability to detect species-specific trophic interactions (reviewed by Symondson 2002, Sheppard and Harwood 2005).

The current study employed a monoclonal antibody-based ELISA to assess the frequency of consumption of forest-floor flies by *Schizocosa* spp. and
A. punctulata. The objective of this research was to quantify differences in resource utilization between these two forest-floor spider species with regards to one group of detrital prey. I tested the hypotheses that both species- and stage-specific variation in fly consumption can result in trophic niche differentiation among generalist predators, and that fly consumption is a function of spider selection rather than prey availability. If variation in resource utilization exists, it could simultaneously promote the coexistence of multiple predator species in the leaf-litter habitat and affect the functional role of each predator species in the forest ecosystem through variable impacts on prey population and community dynamics.

NATURAL HISTORY

The leaf litter layers in an eastern temperate deciduous forest are inhabited by a species-rich generalist predator community including wolf spiders (Araneae, Lycosidae) and their relatives (e.g. Uetz 1976, 1979, Chen and Wise 1999), which exploit a wide range of arthropod prey. Wolf spiders in the genus Schizocosa are among the most common litter-dwelling spiders in deciduous forests throughout the eastern US (Kaston 1948, Dondale and Redner 1978). Three Schizocosa species occur in the study area: S. stridulans, S. ocreata and S. saltatrix. Two of these species, S. stridulans and S. ocreata, are morphologically indistinguishable from one another with the exception of adult males, and juveniles of all species are ambiguous (Stratton 1991), so Schizocosa were considered at the level of genus in this study. In contrast, Anahita punctulata occurs primarily in the southeastern US and is the northernmost representative of the family Ctenidae, which has a predominantly tropical distribution (Peck 1981). Previous distributional data for A. punctulata suggested that the Kentucky-Tennessee border represented the northern edge of its range (Peck 1981); however, recent collections of this species have been made as far north as southern Ohio (Hoffman 2006). Schizocosa and A. punctulata have similar body sizes (Schizocosa females = 8.1 ± 1.21 mm (S. stridulans) (Dondale and Redner 1978, Stratton 1991), A. punctulata females = 8.33 ± 1.60 mm (Peck 1981)). They also coincide spatially and temporally in the forest-floor habitat (Chapter 2) and share similar life history characteristics, with a spring mating period (wandering adult males collected late April – June), overlapping generations with adult females present throughout the spring and summer, and overwintering in the juvenile stage (Dondale and Redner 1978, Peck 1981). Wolf spiders, such as Schizocosa, employ a sit-and-pursue foraging strategy (sensu Schmitz 2007), meaning that they wait at a fixed location until a prey item comes within a critical range, pounce and pursue the prey over a short distance before subduing it (Rovner 1980). Prey detection by wolf spiders is primarily based on visual cues (Persons and Uetz 1998). Little is known about the foraging habits of A. punctulata, though other ctenids are common, nocturnal ambush predators which forage in the litter layer of tropical rainforests (e. g. Gasnier et al. 2002, Joqué 2005).
Previous research has suggested a strong trophic connection between cursorial forest-floor spiders, especially wolf spiders, and litter-dwelling Collembola (Wise and Wagner 1992, Buddle 2002, Wise 2004). Spider predation on Collembola may even elicit trophic cascades which ultimately affect litter disappearance and decomposition rates (Lawrence and Wise 2000, 2004). However, there are other important groups of detritivorous or fungivorous arthropods in the leaf-litter food web which are potentially important resources for spiders, such as forest-floor flies. Increased densities of fungivorous flies and other arthropods, including Collembola, were associated with increased spider densities in the litter layers (Chen and Wise 1999). However, little is known about the importance of flies in the natural diets of cursorial spider species, or about the potential effects of spider predation on fly populations. It is clear that flies are a key prey group for many web-building spiders (Nentwig 1980, 1983, 1985, Nyffeler 1999, Miyashita et al. 2003). The only evidence of cursorial spider predation on adult flies is derived from direct observations of *Pardosa* spp. and *Pirata* spp. (Araneae, Lycosidae) inhabiting agroecosystems (Table 3.1). *Pardosa* spp. have often been observed to consume flies in equal or greater proportions to Collembola (e.g. Nyffeler and Benz 1981, Bardwell and Averill 1997, Morse 1997, Ishijima et al. 2006). In cranberry bogs, flies were the most frequently observed prey items for these spiders representing 33 – 60% of the diets of *Pardosa* spp. (Bardwell and Averill 1997), while in rice paddies flies were considered to be an alternate prey type sustaining spiders when herbivorous insects were not available (Ishijima et al. 2006).

**MATERIALS AND METHODS**

**Sample preparation and ELISA protocol**

In preparation for analysis by indirect ELISA, individual spiders were homogenized in a solution of phosphate-buffered saline (PBS). The dilution ratio selected for each sample; 1:10, 1:20, 1:40 or 1:80 (mg spider:μL PBS), was determined on the basis of spider body weight (<1 to >100 mg) so that the final volume of homogenate obtained was in the range of 40 – 1200 μL. Individual spiders were first placed in clean 1.5 mL microcentrifuge tubes (Fisherbrand™, Fisher Scientific, Pittsburgh, PA), weighed and pulverized in the appropriate amount of PBS. The samples were mixed for 15-20 sec using a mini-vortexer, then centrifuged for 15 min at 8000 g. Finally, the supernatant was extracted, transferred to a clean vial and frozen at -20°C until analysis.

Spiders were analyzed for the presence of fly antigen in their gut contents using the anti-Diptera monoclonal antibody, *DrosW-VI-B8*, and the indirect ELISA protocol developed by Harwood et al. (2007). Prior to analysis, all samples were diluted in PBS to a standard concentration of 1:20,000 (mg spider:μL PBS). Each sample was coated to two adjacent wells of a Fisherbrand™ 96-well clear polystyrene assay plate (Fisher Scientific, Pittsburgh, PA) at a volume of 200 μL per well. Duplicate control wells were used for each sample to control for the effects of non-specific binding on sample absorbance values. The plates were incubated overnight (∼18 hrs) at room temperature after which the samples were
discarded and the plates were washed three times with a solution of PBS-Tween® 20 (0.05% polyethylene glycol sorbitan monolaurate) (Sigma-Aldrich, St. Louis, MO) to remove any non-binding antigen and to block any open binding sites. Next, 200 μL of the DrosW-VI-B8 antibody diluted in PBS-Tween® 20 (1:1000) was added to one of each pair of duplicate wells. The second well received 200 μL of PBS-Tween as a control, and the plates were allowed to incubate for two hours at room temperature. The antibody was then discarded and the plates were again washed three times with PBS-Tween® 20. Subsequently, 200 μL of Immunopure® goat anti-mouse IgG horseradish peroxidase enzyme conjugate (Pierce Biotechnology, Rockford, IL) was added to each well, and the plates were allowed to incubate for one additional hour at room temperature. The conjugate was then discarded and the plates were washed three more times with PBS-Tween prior to the addition of 200 μL of the enzyme substrate o-phenylenediamine (OPD), in a citric acid – phosphate buffer, to each well. The plates were incubated in the dark for 30 minutes at which time 50 μL 2.5 M H2SO4 was added to each well to stop the reaction. The absorbance values were measured using a Thermo Labsystems Multiskan Plus spectrophotometer (Thermo Electron Co., Waltham, MA, USA) at 492 nm. Each assay plate contained thirty-six spider samples, as well as four positive control and eight negative control samples. The positive controls, Drosophila melanogaster Meigen (Diptera, Drosophilidae), were included to ensure that each assay was successful. The negative controls were samples of the organism which showed the strongest reaction to the anti-Diptera antibody in cross-reactivity testing, Graminella nigrifrons (Forbes) (Homoptera, Cicadellidae) (Harwood et al. 2007). The final absorbance values for each spider or control sample was calculated by subtracting the background absorbance caused by non-specific binding (duplicate control wells) from the recorded sample absorbance values. Spiders were considered to be positive for the presence of fly antigen if the sample absorbance value exceeded the mean of the negative control (G. nigrifrons) plus three standard deviations.

**Evaluation of fly antigen decay rates**

The length of time during which prey antigen remains detectable by indirect ELISA in a predator's gut contents can vary significantly between species due to differences in digestive processes (e.g. Symondson and Liddell 1993) or predator body size (Hagler and Naranjo 1997). In order to compare the frequency of fly consumption between Schizocosa spp. and A. punctulata, laboratory feeding assays were used to quantify variation in fly antigen decay rates in adults and juveniles of both species.

Spiders were hand-collected from the forest floor and kept under standardized environmental conditions (22° C, 16:8 L:D photoperiod) for a minimum of two weeks prior to use in the feeding assays. Individual spiders were housed in 8-oz covered plastic dishes with a damp plaster of Paris and charcoal base to maintain high humidity. Water was supplied by a microcentrifuge tube with a cotton wick, and the spiders were provided with an ad libitum supply of early instar house crickets (Acheta domesticus (L.)) every two to
three days. All spiders were subjected to a one-week starvation period prior to use in the feeding assays. At the start of each feeding assay, hungry spiders were transferred to individual Petri plates lined with filter paper and provided with a water source. Vestigial-winged Drosophila melanogaster obtained from laboratory cultures were used as target prey. Juvenile A. punctulata were offered a single D. melanogaster. Hungry wolf spiders were observed to spend a prolonged time consuming a prey item, so juvenile Schizocosa spp. were offered an ad libitum supply of D. melanogaster (3-4 individuals) to encourage more efficient consumption (e.g. Samu and Bíró 1993, Framenau et al. 2000). Feeding was observed for a 3-hr period, at the end of which all uneaten flies were removed from the dishes, and any partially ingested fly remains were extracted from the spiders’ chelicerae. Individuals that did not consume an entire fly, exclusive of macerated indigestible remains, by the end of the feeding period were removed from the assay. Ten spiders were then selected at random and immediately frozen at -20°C. All remaining spiders were transferred to brand new Petri plates, provided with water and given an ad libitum supply of A. domesticus, to mitigate potential effects of starvation on the rate of fly digestion (Symondson and Liddell 1995). The spiders were then placed in an environmental chamber at 22°C with a 16:8 L:D photoperiod for the duration of the experiment. Groups of 10 individuals were selected at random and were frozen at 2, 4, 8, 16 and 24 hrs following the end of the initial feeding period. Spiders were homogenized and screened by indirect ELISA using the methods described above.

Analysis of fly consumption by field-collected spiders

Spiders were collected from the floor of the Berea College Forest from early May through late September of 2007. Collections were conducted over a large tract of land, measuring several hectares and collectors frequently moved to new locations to decrease the potential for any local depletion of spider numbers. All spiders used in the assays were captured between 0700 and 1600 hrs. Large individuals were either located by intensive visual searches of the litter surface in the case of Schizocosa spp., or by searching litter accumulated near the underside of logs for A. punctulata. Small individuals were captured by shaking leaf litter through a 1.5 cm wire mesh screen held over a collecting tub. Spiders were sorted from the fine debris and collected using an aspirator. All spiders were placed in individual vials and kept on ice following capture. Samples were transferred to a battery-powered portable freezer (Engel MT45, Engel USA, Jupiter, FL) within two hours of collection and frozen at -10°C until return to the lab where they were stored in a -20°C freezer until analysis. The gut contents of all field-collected spiders were screened for the presence fly antigen using the monoclonal antibody-based indirect ELISA procedure described above.

Prey availability

The availability of flies and other potential prey (e.g. Collembola) to ground-dwelling spiders was quantified by monitoring prey activity-density on the forest floor. Ten open 8 x 8 m plots were established in each of two 1-ha blocks of
forest located adjacent to the spider sampling areas. Each plot contained two sticky traps designed to capture insects in flight and two pitfall traps to collect arthropods active in the litter. The sticky traps were constructed from 12 x 12 cm squares of stiff metal window screen coated with Tangle Trap® spray adhesive (The Tangle Foot Company, Grand Rapids, MI) and attached to metal stakes oriented vertically just above the litter surface. The pitfall traps consisted of a pair of plastic drinking cups (9 cm diameter) buried flush with the soil surface. The removable inner cups were each fitted with a plastic funnel to deter escape and contained approximately 100 mL 70% EtOH to preserve samples. The traps were exposed for a 48-hour period every 2-3 weeks from early May through late September.

**Statistical analyses**

Species- and stage-specific differences in fly consumption by spiders were analyzed using a $\chi^2$ analysis. Weekly spider collections were pooled for analysis of seasonal trends in fly consumption. Correlations between fly consumption and prey availability in the litter layer were assessed using a multiple logistic regression model. Data were log transformed when necessary to achieve normality and homogeneity of variances.

**RESULTS**

**Evaluation of fly antigen decay rates**

One hundred percent of individuals of both species assayed immediately following ingestion of fruit flies (time = 0 hrs) tested positive for the presence of fly antigen (Figure 3.1). However, there were significant differences in the initial absorbance values, and hence the amount of detectable fly antigen, in the gut contents of the two species. Juvenile *Schizocosa* spp., which consumed flies *ad libitum* throughout the feeding period, had absorbance values six times higher than those of *A. punctulata* which were only offered a single fly. There were also species-specific differences in the rate of antigenic decay. Fly antigen decayed exponentially in *Schizocosa* spp. over the 24-hr period ($y = 0.4851e^{-0.137x}$, $r^2 = 0.9748$), but decay followed a logarithmic model for *A. punctulata* ($y = -0.0196\ln(x) + 0.069$, $r^2 = 0.6701$) (Figure 3.1). There was one outlier ($y=0.273$ at time = 8 hrs), which was excluded from *A. punctulata* model. The detection intervals, or the approximate duration of time during which the antigen can be detected post-ingestion, were calculated as the intersection between the regression curve and the positive threshold lines. Juvenile *Schizocosa* had a detection period of 20.3 hrs with an antigenic half-life, defined as the time at which half of the detectable antigen present in the spiders gut contents has disappeared, of 3.9 hrs. Juvenile *A. punctulata* had a much shorter detection period of only 8.5 hrs, and an antigenic half-life of 3.4 hrs. The proportion of individuals testing positive for fly antigen decreased over time in a similar manner as the mean absorbance values for both *Schizocosa* spp. ($y = 123.95e^{-0.0597x}$, $r^2 = 0.81$) and *A. punctulata* ($y = -26.26\ln(x) + 83.456$, $r^2 = 0.80$) (Figure 3.1). At the
end of the 24-hr assays, 20% of Schizocosa spp. and 13% of A. punctulata individuals still tested positive for the presence of fly antigen.

**Analysis of fly consumption by field-collected spiders**

Gut contents of 948 individual Schizocosa spp. and 947 A. punctulata were screened for the presence of fly antigen using indirect ELISA (Table 3.2). The overall frequency of fly consumption was similar for both species, with a total of 8.6% Schizocosa spp. and 8.2% A. punctulata, testing positive for fly antigen by indirect ELISA ($X^2 = 0.16, p = 0.70$). There were strong seasonal trends in fly consumption, with peaks in frequency of positive individuals occurring in spring and fall for both species (Figure 3.2). There was no evidence of stage- or gender-specific variation in fly consumption by Schizocosa spp. ($X^2 = 0.07, p = 0.97$). There was, however, a significant difference in fly consumption by adult female and juvenile A. punctulata. Approximately 18.5% of adult and penultimate females (n = 124) tested positive for fly antigen compared to only 6.7% of juveniles (n = 821) ($X^2 = 22.74, p < 0.0001$).

A logistic regression was used to evaluate the effects of spider size (body mass) and developmental stage on fly consumption. There was no significant effect of spider size on frequency of fly consumption by A. punctulata ($X^2_{896} = 890.88, \text{deviance} = 486.88, p = 0.29$), but there was a significant effect of stage (p = 0.0001). Adult female A. punctulata tested positive for fly antigen twice as frequently as did juveniles. In the case of Schizocosa spp., spider body weights exhibited a bivariate distribution, as numbers of spiderlings (< 2.0 mg) and large adults (>50 mg) assayed were high, but numbers of intermediate-sized juveniles collected were relatively low. Therefore individual Schizocosa were placed in weight categories prior to regression analysis. Four categories were used: spiderlings ≤ 2.0 mg, juveniles = 2.1 – 20.0 mg, large juveniles and small adults = 20.1 – 50.0 mg, and large adults (mostly females) = > 50.0 mg. There was no significant effect of spider stage or sex on Schizocosa consumption of flies ($X^2_{893} = 892.96, \text{deviance} = 525.66, p = 0.38$), but there was a significant difference in fly consumption among size classes. Juvenile spiders weighing between 2.1 and 20.0 mg tested positive for fly consumption 1.5 – 2.5 times more frequently than all other size classes (p = 0.004).

**Prey availability**

Sticky screen traps were used to monitor seasonal trends in the flight activity of flies within 12 cm of the litter surface. This trapping method was useful for capturing small gnats and midges (Nematocera) which comprised over 97% of the total catch during the sampling period. Brachycera represented a very small fraction (< 3%) of the flies caught, which may reflect a combination of factors including lower densities, differing microhabitat utilization, or higher frequency of escape from the trap by larger and stronger flies. The fungus gnats (Sciaridae) were the dominant group of Nematocera captured in the sticky traps, comprising >90 % of total capture. The gall midges (Cecidomyidae) were > 8% of the Nematocera, and other gnats and midges represented very small fractions of the total capture. There was a strong seasonal trend in Nematocera flight activity on
the forest floor, with peak captures occurring mid-May through mid-June (Figure 3.3). Pitfall traps containing ethanol also proved useful to monitor seasonal changes in relative activity of many detrital fly species, such as large Brachycera, which were not well represented in the sticky trap samples. In pitfall trap captures, Brachycera were the dominant group collected (> 76% total), and the largest proportion of these flies were in the superfamily Muscoidea, though scuttle flies (Phoridae) and fruit flies (Drosophilidae) also represented significant proportions of the total flies captured. Pitfall trap captures confirmed seasonal fungus gnat (Sciaridae) activity patterns derived from sticky trap captures, with peak numbers of individuals captured in May and June, but limited activity later in the summer and fall (Figure 3.3). The relative proportion of Brachycera was low in the pitfall trap captures through much of the summer, but there was a significant increase in the number of Muscoidea captured in the fall near the end of the sampling period (Figure 3.3).

Pitfall traps were also used to monitor the seasonal availability of other litter-dwelling prey taxa. Collembolans were the dominant group of detritivores collected in the traps, and more than 86% of the total Collembola belonged to the families Tomoceridae and Entomobryidae. Tomoceridae alone accounted for 70% of the total collembolans captured during the study period. Other common families collected were Isotomidae and Sminthuridae, each accounting for < 7% of the total individuals captured. A fifth family, Hypogastruridae occurred rarely in the traps with the exception of occasional outbreaks, when hundreds of individuals could be found in a single trap. Sminthuridae and Hypogastruridae often secrete defensive compounds which make them unpalatable as prey (Bitzer et al. 2004), so they will not be considered here. These species will not be considered further due to infrequent occurrence of high densities. The dominant group of Collembola, the Tomoceridae, exhibited strong seasonal variation with greatest numbers of individuals captured early in the spring, followed by a continuous decline in average activity-density over the course of the summer and fall (Figure 3.4). Other potential prey groups common in the pitfall samples were field and house crickets, *Gryllus* sp. and *Acheta domesticus* (Orthoptera, Gryllidae), respectively, and wood roaches, *Parcoblatta* spp. (Blattodea, Blattellidae). In all cases, macroarthropod activity-density was relatively low until mid-July at which time activity increased and peaked in late summer – early fall (Figure 3.4).

A multiple logistic regression model was also used to determine whether prey availability was correlated with fly consumption for both flies and alternate prey. Since prey surveys were conducted every 2-3 weeks gut content data from 14-day intervals were pooled for the analysis. In the first analysis gut content data were compared to sticky trap captures of fly. There was no significant effect of fly availability on consumption for either *Schizocosa* (*χ^2^ 877 = 880.85, p = 0.13* (Nematocera), *p = 0.25* (Brachycera) or for *A. punctulata* (*χ^2^ 739 = 738.88, p = 0.09* (Nematocera), *p = 0.38* (Brachycera). Prey activity data from pitfall traps was used to fit a second logistic regression model, which revealed no significant effect of any alternate prey species (Collembola, wood roaches, crickets) on frequency of fly consumption by *A. punctulata* (*χ^2^ 568 = 573.08, p > 0.10).
Consumption of flies by *Schizocosa* was marginally correlated with the activity-density of two alternate prey species, wood roaches and house crickets, but not with Collembola (χ²₆₈₂ = 624.21, p = 0.02 (wood roaches), p=0.04 (crickets), p = 0.76 (Tomoceridae)).

**DISCUSSION**

*Evaluation of fly antigen decay rates*

Quantification of antigen decay rates and detection periods not only allows for comparisons of variation in prey consumption among different predator species, but also aids in the interpretation of the data obtained from analyses of field-collected individuals. The detection periods observed for juvenile *Schizocosa* spp. were approximately 6-8 hrs longer than was previously recorded for sheetweb spiders (Linyphiidae) using the same antibody (Harwood et al. 2007), though the antigenic half-lives were very similar (3.5-5 hrs). The detection period calculated for juvenile *A. punctulata* was significantly shorter than *Schizocosa* spp. and the linyphiids, but with a similar antigenic half-life of 3.4 hours. This implies similar rapid antigen decay in the digestive tracts of all species immediately following ingestion. However, wolf spiders, which may experience frequent resource limitation in nature (Wise 1993), probably store partially digested prey in gut diverticulae for an extended period of time (Nakamura 1977, Foelix 1996). This could explain the prolonged detection interval for these spiders, and is supported by the observation that 20% of *Schizocosa* spp. still tested positive for the presence of fly antigen 24 hrs after ingestion. The relatively short detection interval measured for *A. punctulata* is most likely an artifact of the extremely low initial absorbance values measured for this species immediately following fly consumption (Figure 3.1) which may have been caused by antigen dilution.

The total amount of fly antigen in a spider’s gut contents is dependent not only on the length of time since consumption, but also on the number of flies ingested and the size of individuals eaten. Indirect ELISA can be insensitive when the proportion of target antigen in a sample is very small due to competition for limited binding sites on the assay plate (Hagler et al. 1997, Hagler 1998). The mean absorbance value measured for juvenile *A. punctulata* screened immediately following ingestion of *D. melanogaster* was significantly lower (OD = 0.09 ± 0.03 at 492 nm) than the mean value recorded for juvenile *Schizocosa* spp. (OD = 0.57 ± 0.08 at 492 nm). This is consistent with the larger predator to prey body size ratios for *A. punctulata* which weighed on average 10.2 ± 3.86 mg, compared to *Schizocosa* spp. which had a mean body weight of 4.12 ± 0.83 mg. Additionally, *Schizocosa* spp. were offered multiple prey items and may have ingested significantly more fly antigen than did *A. punctulata*. In order to determine whether the amount of prey ingested had a significant effect on the ELISA response for juvenile *Schizocosa*, spiders were weighed prior to, and immediately following the 3-hr feeding period. Individual spiders gained an average of 0.47 ± 0.04 mg during the experimental feeding period with a range of 0 - 1.4 mg. Since all spiders were observed to eat at least one fly, lack of weight...
gain by individual spiders is assumed to result from excretion during the feeding period. There was no correlation between spider weight gain due to fly ingestion and the absorbance values recorded for spiders frozen immediately after feeding, indicating that this amount of variation in antigen volume was insufficient to affect the sensitivity of the ELISA. In the forest, prey selection by spiders is likely to be dependent on predator to prey body size ratios (Wise 1993), and small size of flies presented to A. punctulata in the laboratory assay may not be representative of a typical predation event in nature. Strong positive ELISA responses measured for large field-collected spiders likely result from ingestion of fly species larger than D. melanogaster.

The length of time during which the indirect ELISA was able to detect fly antigen in the gut contents of spiders using the DrosW-VI-B8 antibody was relatively short (<24 hrs) compared to other similar monoclonal antibody systems in which detection periods can range from several days to more than a week (e.g. Symondson and Liddell 1995, Symondson et al. 1999a,b, Harwood et al. 2001b, 2004). Extended detection periods can increase the probability of detecting infrequent predation events, but make it difficult to interpret the gut content data from field-collected predators because a strong positive response could result from a single, recent predation event or from multiple predation events occurring anytime during the detection interval. In the current study, spiders testing positive for fly antigen are presumed to have eaten at least one fly in the 20 hours prior to collection. Since wolf spiders may exhibit prey consumption rates as low as a single prey item per day in nature (Nyffeler and Benz 1988, Nyffeler and Breene 1990), positive ELISA responses obtained for individual Schizocosa and A. punctulata likely result from consumption of a single fly.

**Analysis of fly consumption by field-collected spiders**

Prey availability and encounter and capture rates may be important determinants of dietary composition for highly polyphagous predators, such as spiders, which often persist under conditions of resource limitation in nature (Riechert and Lockley 1984, Wise 1993). The gut content analysis revealed that approximately 8% of the Schizocosa and A. punctulata collected from the forest floor had recently ingested one or more flies. Peak Diptera consumption by both species (>25%) occurred in early June, coincident with seasonally high activity of small Nematocera near the litter surface in late spring and early summer. Most of the Diptera captured during this period were dark-winged fungus gnats (Sciaridae), and adult activity virtually ceased in mid-June following the primary reproductive period. Despite the apparent temporal correlation between the early-season peak in Diptera consumption and the gnat flight activity period, there was no statistically significant relationship between Diptera consumption and availability measured over the course of the summer. This is not to suggest that the high frequency of Diptera consumption by spiders in spring was completely unrelated to Diptera activity, but rather that continuous spider predation on Diptera throughout the summer and fall was not correlated with similar levels of fungus gnat activity later in the season. Variability in Diptera consumption during the
summer and fall may be related to densities of larger Diptera, including most Brachycera, which were observed pitfall trap captures but were not caught in the sticky traps used to monitor Diptera flight activity. Unfortunately the trapping methods used did not generate enough data on Brachycera dynamics to test this hypothesis.

Collembola and Diptera are the two prey taxa most frequently encountered in observational studies of wolf spider predatory habits (Table 3.1). Collembola, particularly large litter-dwelling species of Tomoceridae and Entomobryidae, were considered to be preferred prey for the cursorial spiders in this study due to their high nutritional value (Toft and Wise 1999) and indirect implications of their importance in the diet of Schizocosa in the forest-floor food web (e.g. Wise 2004). It was hypothesized that if Diptera serve as an alternate resource for forest-floor spiders, frequency of consumption should be negatively correlated with the availability of preferred tomocerid Collembola. The results of the spider gut content assays clearly did not support this hypothesis. Tomocerid activity-density was highest in the spring and decreased steadily throughout the summer into the fall so that peak tomocerid activity-density occurred concurrently with peak fungus gnat flight activity and peak rates of Diptera consumption. Decreased tomocerid activity-density was not correlated with increased Diptera consumption in late summer and early fall. There was a marginally significant positive correlation between Diptera consumption by Schizocosa and activity-density of common litter-dwelling macroarthropods (crickets and roaches) resulting from the co-occurrence of mid-summer increases in macroarthropod numbers and summer and fall peaks in spider predation on Diptera. The results of this analysis indicate that there is a significant seasonal shift in resource availability for forest-floor spiders, with Collembola and small gnats (Nematocera) which are dominant in the spring and early summer, giving way to larger Diptera and litter-dwelling macroarthropods in late summer and early fall. There were however, no clear correlations between Diptera consumption by Schizocosa and A. punctulata and any measure of prey availability indicating that Diptera consumption by these spiders is driven by mechanisms other than simple prey encounter rates.

Wolf spiders generally require a diet composed of multiple prey types in order fulfill nutritional requirements and attain maximum fitness (Uetz et al. 1992, Toft and Wise 1999a, Oelbermann and Scheu 2002a). Laboratory studies have demonstrated that the addition of Diptera (Drosophila sp.) to a diet consisting of high quality collembolans, results in increased spider fitness even when both prey groups were reared on the same basal resource (Toft and Wise 1999a). Conversely dark-winged fungus gnats (Sciaridae) are poor quality prey which lead to development of aversion behavior in Schizocosa (Toft and Wise 1999a,b). Spiders may have the ability to actively select prey items on the basis of quality to maintain balance of amino acids or essential nutrients (e.g. Greenstone 1979, Toft 1999, Mayntz et al. 2005, particularly when resources are readily available (Riechert and Luczak 1982). It is therefore possible that spiders may actively select Diptera in addition to other components of the diet to fulfill
nutrient requirements particularly in spring when collembolans are readily available.

**Species- and stage-specific variation in fly consumption**

The gut content survey suggested that there were no species-specific differences in the frequency or occurrence of fly consumption by juvenile spiders. Likewise, there were no differences in fly consumption between adult *Schizocosa* spp. and juveniles of either species. The only evidence of variation in utilization of this prey group was seen in adult female *A. punctulata*, which tested positive for fly antigen significantly more frequently than both juveniles of this species and all stages of *Schizocosa* spp. This shift in resource utilization may be related to ontogenetic changes in nutritional requirements, or may simply be related to variation in prey encounter rates resulting from shifting foraging behaviors or habitat use. Kruse et al. (2008) documented higher rates of fly predation for nocturnal ground-dwelling predators, than for diurnal wolf spiders in a controlled laboratory experiment. The observed patterns were due to differences in foraging success rather than encounter rates. Wolf spiders encountered flies more frequently on the substrate, but captured fewer individuals than did predators foraging at night when temperatures were below the flight threshold for the flies, thus deterring escape. While the foraging habits of adult *A. punctulata* have not been documented, most Ctenidae are nocturnal hunters (Gasnier et al. 2002, Joqué 2005). Nocturnal foraging on the litter surface when temperatures are low could account for the higher frequency of Diptera consumption by female *A. punctulata* compared to *Schizocosa* which primarily forage during the day (Cady 1984). Small juvenile *A. punctulata* and *Schizocosa* spp. are likely to forage lower in the litter profile than adult spiders (Wagner et al. 2003) and thus may encounter fewer adult flies alighting on the litter surface. However, the anti-Diptera monoclonal antibody used in this study reacted to both adult and larval flies (Harwood et al. 2007). Juvenile spiders may therefore consume fly larvae which inhabit the lower organic or mineral horizons in the forest floor (Frouz 1999, Hövemeyer 1999).

The immunoassay-based gut content analysis used in this study provided semi-quantitative data on the consumption of one prey group found in the forest-floor food web. While this method allows for comparisons of resource utilization among species, it does not permit any assessment of the importance of flies in the diets of forest-floor spiders relative to other prey types in the absence of data on prey consumption rates, and other prey consumed. Previous observational studies have suggested that flies are a significant component of the diet of wolf spiders in agroecosystems (Table 3.1). Likewise, flies may be an important component of the diet of forest-floor spiders, though further evidence is required to support this conclusion.
Table 3.1 Summary of studies documenting direct observations of arthropod predation by wolf spiders (Araneae, Lycosidae)

<table>
<thead>
<tr>
<th>Species</th>
<th>System</th>
<th>Total</th>
<th>Araneae</th>
<th>Collembola</th>
<th>Diptera</th>
<th>Hemiptera</th>
<th>Hymenoptera</th>
<th>Lepidoptera</th>
<th>Reference</th>
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<tbody>
<tr>
<td><em>Pardosa</em> spp.</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td><em>P. amentata</em></td>
<td>Forest</td>
<td>46</td>
<td>11</td>
<td>13</td>
<td>67</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>Edgar 1970*</td>
</tr>
<tr>
<td><em>P. chelata</em></td>
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<td>210</td>
<td>34</td>
<td>6</td>
<td>17</td>
<td>3</td>
<td>1</td>
<td>4</td>
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</tr>
<tr>
<td><em>P. floridana</em></td>
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<td>6</td>
<td>16.7</td>
<td>33.3</td>
<td>33.3</td>
<td>16.7</td>
<td>–</td>
<td>–</td>
<td>Bardwell and Averill 1997</td>
</tr>
<tr>
<td><em>P. lapidicina</em></td>
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<td>13</td>
<td>–</td>
<td>54</td>
<td>31</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Morse 1997</td>
</tr>
<tr>
<td><em>P. lugubris</em></td>
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<td>119</td>
<td>24</td>
<td>2</td>
<td>33</td>
<td>28</td>
<td>4</td>
<td>5</td>
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<tr>
<td><em>P. lugubris</em></td>
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<td>31</td>
<td>3</td>
<td>39</td>
<td>26</td>
<td>–</td>
<td>3</td>
<td>–</td>
<td>Nyffeler and Benz 1981*</td>
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<td><em>P. moesta</em></td>
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<td>20</td>
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<td>60</td>
<td>–</td>
<td>–</td>
<td>20</td>
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</tr>
<tr>
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<td>76</td>
<td>3</td>
<td>38</td>
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<td>5</td>
<td>12</td>
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<td>Hallander 1970*</td>
</tr>
<tr>
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<td>–</td>
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<td>35</td>
<td>7</td>
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<td>12.3</td>
<td>9.2</td>
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<td>–</td>
<td>78</td>
<td>–</td>
<td>–</td>
<td>Kiritani et al. 1972*</td>
</tr>
<tr>
<td><em>P. pseudoannulata</em></td>
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<td>59.4</td>
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<td>–</td>
<td>Ishijima et al. 2006</td>
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<td>7.7</td>
<td>Agnew and Smith 1989</td>
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</tr>
<tr>
<td><em>P. piraticus</em></td>
<td>Salt marsh</td>
<td>264</td>
<td>22</td>
<td>43</td>
<td>12</td>
<td>11</td>
<td>–</td>
<td>–</td>
<td>Schaefer 1974*</td>
</tr>
<tr>
<td><em>P. subpiraticus</em></td>
<td>Rice paddy</td>
<td>39</td>
<td>7.7</td>
<td>–</td>
<td>15.4</td>
<td>64.1</td>
<td>–</td>
<td>–</td>
<td>Ishijima et al. 2006</td>
</tr>
</tbody>
</table>

* Previously reviewed by Nentwig (1986).
Table 3.2 Percentage of field-collected spiders testing positive for fly antigen using indirect ELISA

<table>
<thead>
<tr>
<th>Stage</th>
<th>Schizocosa spp. (n)</th>
<th>Anahita punctulata (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td></td>
<td></td>
</tr>
<tr>
<td>♀'s</td>
<td>9.1 (230)</td>
<td>22.6 (84)</td>
</tr>
<tr>
<td>♂'s</td>
<td>9.3 (140)</td>
<td>0.0 (2)</td>
</tr>
<tr>
<td>Total</td>
<td>9.2 (370)</td>
<td>22.1 (86)</td>
</tr>
<tr>
<td>Juveniles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sub-adult ♀′s</td>
<td>0.0 (8)</td>
<td>10.0 (40)</td>
</tr>
<tr>
<td>Immatures</td>
<td>8.6 (570)</td>
<td>6.7 (821)</td>
</tr>
<tr>
<td>Total</td>
<td>8.5 (578)</td>
<td>6.8 (861)</td>
</tr>
</tbody>
</table>
Figure 3.1 Rate of fly antigen decay in the digestive tracts of juvenile spiders.
Figure 3.2 Seasonal trends in the proportion of a) Schizocosa spp. and b) Anahita punctulata testing positive for the presence of fly antigen by indirect ELISA. Dashed lines represent 95% confidence intervals.
Figure 3.3 Seasonal activity-density of adult flies a) in flight above the litter surface and b) in the litter layer.
Figure 3.4 Seasonal activity-density of litter-dwelling a) Collembola and b) macroarthropods (wood roaches and crickets).
CHAPTER IV

Forest-floor food web structure: Stable isotope ($\delta^{15}$N and $\delta^{13}$C) analysis reveals resource utilization by generalist predators

SUMMARY

Traditionally, food web research has focused on the aboveground component of the grazing web with little consideration of detrital food web structure or the integration of detrital and grazing webs above the level of basal resources. However, recent interest in understanding the role of community-level dynamics in regulating ecosystem function has spurred research on detrital food web structure and dynamics. Elucidating the degree to which complex food webs are compartmentalized into distinct grazing and detrital energy channels can also lead to a better understanding of how species interactions, such as subsidized trophic cascades involving generalist predators, may affect ecosystem dynamics. The objective of this study was to describe the structure of the leaf-litter food web of a temperate deciduous forest and to quantify the strength of predator-mediated connections between the detrital and aboveground-grazing webs in this system.

Stable isotope analysis ($\delta^{13}$C and $\delta^{15}$N) was used to test the hypotheses that 1) the leaf-litter food web is characterized by extensive omnivory at all trophic levels including generalist predators (i.e. IGP) and 2) litter-dwelling spiders are highly polyphagous predators which function to link the detrital and grazing energy channels via consumption of herbivorous insect prey. Spider and prey nitrogen signatures confirmed the frequent occurrence of omnivory amongst detritivorous and fungivorous arthropods as well as IGP within the spider community. Three distinct trophic groups were identified based on $\delta^{15}$N values: specialist consumers of fungal hyphae (Tomoceridae), generalist consumers of detritus and associated microbes (macro- and mesoarthropods) and predators (spiders and centipedes). Flies, however, were highly enriched in $^{15}$N relative to all other arthropods (including predators). Arthropods associated with detrital resource were significantly enriched in $^{13}$C than were primary consumers from the grazing web. A single-isotope ($\delta^{13}$C) mixing model (IsoError) revealed that the majority of litter-dwelling spiders derive most of their energy from the decomposition subweb. Thus predator-mediated links between grazing and detrital subwebs were relatively weak in this system. This supports the idea that the two subwebs are best viewed as distinct compartments of the aboveground forest food web.
INTRODUCTION

Historically, much research on terrestrial food webs has focused on describing the structure of aboveground grazing webs and understanding how interactions among plants, herbivores and predators may be related to primary productivity (e.g. Power 1992). However in most terrestrial ecosystems, such as temperate forests, a significantly greater proportion of energy derived from net primary production is transferred directly to the detrital food web than is consumed by herbivores (Wiegert and Owen 1971, Hairston and Hairston 1993, Odum and Biever 1984, Polis and Strong 1996). Soil and litter fauna, which function both as primary decomposers of detritus and as consumers of saprophytic microbes, play an important role in regulation of decomposition and mineralization processes (Seastedt 1984, Moore et al. 1988, Verhoef and Brussard 1990). Ground-dwelling generalist predators, such as spiders, may also play an integral role in detrital food web dynamics and ecosystem processes by limiting populations of detrital prey and eliciting trophic cascades which can influence decomposition and mineralization (e.g. Kajak et al. 1991, Kajak 1997, Lawrence and Wise 2000, 2004). Therefore, elucidating detrital food web structure and the role of generalist predators in soil and litter communities, is essential to developing a more comprehensive understanding of ecosystem function.

The idea that grazing and detrital webs represent two distinct channels, or pathways of energy flow within an ecosystem has permeated terrestrial food web research (e.g. Teal 1962, Odum 1969, Odum and Biever 1984, Moore et al. 2004). However this dichotomy is valid only at the level of primary consumers, as omnivores and polyphagous predators often utilize resources from both webs (Pimm and Lawton 1980, Pimm 1982, Moore and Hunt 1988, Polis and Strong 1996). Predator utilization of resources from outside of the primary energy channel (i.e. prey subsidies) may have significant effects on predator and prey dynamics within the primary food web, possibly resulting in increased predation pressure and limitation of in situ prey (e.g. Polis and Strong 1996). Additionally, the predator-mediated coupling of the grazing and detrital webs may have significant implications for ecosystem stability (Moore et al. 2004, McCann et al. 2005, Rooney et al. 2006).

The current study focuses on a diverse community of ground-dwelling spiders inhabiting the litter layer of a temperate deciduous forest. The forest ecosystem is characterized by vertical stratification of the habitat into canopy, mid- and understory vegetation and forest-floor layers. Herbivores that forage in the canopy or mid-story are primarily accessible to ground-dwelling generalist predators when they fall or drop from plants (e.g. Pringle and Fox-Dobbs 2008). Spider species that engage active in hunting behaviors on the litter surface are more likely to encounter these herbivores than less mobile species. Spiders with broad habitat domains (sensu Preisser et al. 2007), specifically species that can climb into the understory vegetation, are likely to encounter and consume more herbivores than spiders that forage exclusively in the litter layers. Predator life history traits may also affect trophic
interactions within the detrital web. For example, large cursorial species are more likely to engage in intraguild predation, and therefore occupy higher trophic levels than small species or juvenile spiders. Conversely sedentary web-building species are unlikely to encounter spiders or other predators (Wise 1993) and therefore occupy the lower end of the predator trophic spectrum. The primary objectives of this research were to use natural variation in consumer stable isotope ratios ($\delta^{15}$N and $\delta^{13}$C) to 1) quantify the structure of the leaf-litter food web with emphasis on the species-specific trends in resource utilization within the generalist predator community, and 2) examine predator-mediated trophic connections between the grazing and detrital energy channels. I tested the hypotheses that 1) the leaf-litter web is characterized by frequent omnivory, including intraguild predation, which results in lack of clearly defined trophic levels, and 2) spiders function to link the aboveground grazing web and the detrital web via consumption of herbivorous insect prey.

**Stable isotope techniques in food web research**

The relatively recent adaptation of stable isotope techniques for use in ecological research has provided researchers with a valuable tool for studying trophic organization in complex food webs. Analysis of natural variation in plant and animal stable isotope ratios ($\delta^{15}$N and $\delta^{13}$C) simultaneously provides information about trophic structure and resources assimilated over time (Peterson and Fry 1987). Consumers are predictably enriched in $^{15}$N relative to resources, as a result of nitrogen fractionation occurring during digestion, assimilation or excretion of food (DeNiro and Epstein 1981, Minagawa and Wada 1984, McCutchan et al. 2003). Thus trophic-level enrichment in consumer $\delta^{15}$N values can be used to estimate positions of diverse consumers relative to basal resources in complex food webs, including detrital systems (e.g. Ponsard and Arditi 2000, Scheu and Falca 2000). Consumer $\delta^{13}$C values are similar to the $\delta^{13}$C values of their resources, as trophic fractionation of carbon by animals is typically minimal (DeNiro and Epstein 1978, McCutchan et al. 2003). Thus $\delta^{13}$C values can be used to trace carbon flow from isotopically distinct resources to generalist predators, such as spiders, both within and between food webs (e.g. Akamatsu et al. 2004, Kato et al. 2004, Briers et al. 2005).

Analysis of natural variation in consumer stable isotope ratios has not been frequently used to compare grazing and detrital resources (but see Wise et al. 2006). Thus far there has been relatively little research devoted to documenting the effects of decomposition on stable isotope ratios, though there is a general trend towards a slight increase in decomposed litter $\delta^{13}$C values compared to fresh litter or live plants ($\approx 2\%$) (Melillo et al. 1989, Wedin et al. 1995). The effects of fungal colonization on litter $\delta^{13}$C values are also slightly ambiguous. Ruess et al. (2005) observed either slight enrichment or depletion in hyphal $\delta^{13}$C relative to leaf litter for ascomycete and mitosporic species of soil fungi, respectively. However, saprophytic basidiomycetes,
more commonly found in leaf litter and on woody debris, are significantly enriched in $^{13}$C relative to litter (Gleixner et al. 1993, Hobbie et al. 2001). The combination of decomposition and microbial colonization is predicted to result in some level of $^{13}$C-enrichment of decaying leaf litter compared to foliage, though the magnitude of the shift may be relatively small (Park and Lee 2006). This study quantified variation in $\delta^{13}$C values among forest-floor herbivores and detritivores to test the general hypothesis that stable isotope analysis can be used to distinguish between grazing and detrital resources in a system with a diverse plant community.

MATERIALS AND METHODS

Sample collection
Spiders and prey were collected from the leaf litter of the Berea College Forest during the spring and summer of 2006 and 2007. Twenty-six commonly encountered species or genera representing fifteen families of litter-dwelling cursorial and web-building spiders were included in this study. In addition, two other abundant forest-floor predators, harvestmen (Arachnida, Opiliones) and centipedes (Chilopoda), as well as foliage-dwelling orb-weaving spiders (Araneae, Araneidae) were analyzed for comparison. Table 4.1 summarizes the foraging habits and life history characteristics of the spiders included in this study. Large surface-active spiders were located by visual searches of the litter, collected by hand and placed in individual vials. All other spiders were collected by shaking leaf litter through a large sifting screen (15 mm mesh) held over a plastic tub. Spiders were sorted from the fragmented litter and debris in the tub, collected by hand or with an aspirator and placed in individual vials. Potential prey items, including Collembola, crickets, wood roaches, termites, millipedes, moth larvae and flies were also collected from the litter layers using the sifting method. Herbivorous insects were dislodged from the understory vegetation by beating branches over a large plastic tub, or were gathered from the litter layer with detrital prey. Flying insects were captured by sweeping a net just above the litter surface. Spiders and prey were either placed on ice or frozen in the field and returned to the lab for identification and processing. All samples were stored frozen (-20°C) until prepared for analysis.

Stable isotope analysis
In preparation for analysis, all arthropods were oven-dried at 60°C for 24-48 hours. Spiders were identified to the lowest possible taxonomic classification, weighed (dry weight) and measurements were taken of body length and carapace width. All arthropod tissue was ground to a fine powder using a mortar and micro-pestle or ball mill. A minimum of 1 mg prepared tissue was required for analysis, so small organisms, such as Collembola, were pooled to produce adequate sample weights (2-20 individuals per sample). Large organisms, such as adult wolf spiders, were homogenized and a 1-2 mg
subsample was collected from each individual. Prepared samples were weighed and packed into 5 x 9 mm tin capsules (Costech Analytical Technologies Inc., Valencia, CA) for analysis. Stable isotope ratios (δ¹³C and δ¹⁵N) for spiders and prey were determined using a PDZ-Europa elemental analyzer coupled to a PDZ-Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Analyses were done by the Stable Isotope Research Facility at the University of California-Davis. Stable isotope values are expressed in δ units or the isotopic ratio relative to a known standard using the equation

\[
\delta X = \left( \frac{R_{sample}}{R_{std}} - 1 \right) \times 1000
\]

where δX (‰) = δ¹³C or δ¹⁵N, R=¹³C/¹²C or ¹⁵N/¹⁴N and the standards are Pee Dee Belemnite for δ¹³C and atmospheric nitrogen for δ¹⁵N.

**Spider biomass**

The biomass of each spider taxon in the leaf litter was estimated as

\[
Biomass = \left( D_F \times M_F \right) + \left( D_J \times M_J \right)
\]

where D is the seasonal mean spider density, M is mean individual body mass (dry weight), F denotes female values and J denotes juveniles. Spider densities were based on data from litter samples collected during the summer of 2006 (Chapter 2). However, the litter sifting method used to gather this data was relatively ineffective for sampling densities of larger spiders, including adult *Schizocosa, A. punctulata, A. virgo, G. fontinalis* and *Drassyllus* spp. as these spiders often escaped (e.g. *Schizocosa*) during the litter collection or were not active during the day (e.g. *A. virgo*). However, all stages of these species were captured in pitfall traps, so trap data was used to estimate the ratio of females to juveniles present in forest for each species. The biomass estimates for these species were calculated as

\[
Biomass = \left( \frac{P_F \times D_J}{P_J} \right) M_F + \left( D_J \times M_J \right)
\]

where P = mean spiders per trap. In the case of a few species including, *Phidippus whitmani* (Salticidae), *Pisaurina mira* (Pisauridae), *Castianeira cingulata* (Corinnidae), *Ariadna bicolor* (Segestriidae) and other large web-builders, adult spiders were not frequently collected in either pitfall traps or litter samples. Therefore no biomass estimates are presented for adults of these species. The biomass estimates derived for spiders included in this did
not include adult males as they are typically present in the forest for a short period of time and are likely searching for mates rather than foraging.

**Statistical analysis and modeling**

Variation in $\delta^{15}N$ and $\delta^{13}C$ between years and among spider and prey groups was assessed using a mixed model (SAS 8.01, SAS Institute, Cary, NC). Multiple comparisons were used to explore significant effects, with p-values adjusted using the false discovery rate method (Benjamini and Hochberg 1995). Data were log-transformed as necessary to improve normality and fit of the model. The contribution of carbon originating in the grazing subweb to the diet’s of ground-dwelling spiders was assessed using a two-source, single isotope mixing model, IsoError (Phillips and Gregg 2001). The IsoError model was selected because the two sources considered in this analysis, herbivores and detritivores, embody a wide array of prey organisms, all of which differ in feeding modes, physiology and resource utilization. Therefore, a significant amount of variation in source $\delta^{13}C$ values is expected. Unlike other mixing models, the IsoError procedure allows us to account for source variation in $\delta^{13}C$ values, as well as for consumer variance (Phillips and Gregg 2001).

Prior to input in the model, spider $\delta^{13}C$ values were corrected for trophic-level carbon fractionation using the equation

$$\delta^{13}C_{\text{corrected}} = \delta^{13}C_{\text{spider}} - \Delta^{13}C \ (\text{TL-1})$$

where $\Delta^{13}C$ is the trophic level fractionation value for carbon $\approx 0.4$ (Oelbermann and Scheu 2002b), and TL is spider trophic level and is determined by the equation

$$\text{TL} = \lambda + \left(\delta^{15}N_{\text{spider}} - [\delta^{15}N_{\text{herbivore}} \times \alpha + \delta^{15}N_{\text{detritivore}} \times (1-\alpha)]\right)/\Delta^{15}N$$

where $\lambda$ is the trophic level of the baseline organisms ($\lambda=2$ for primary consumers) and $\alpha$ is the proportion of nitrogen obtained from the grazing food web, calculated as

$$\alpha = (\delta^{13}C_{\text{spider}} - \delta^{13}C_{\text{detritivore}}) / (\delta^{13}C_{\text{herbivore}} - \delta^{13}C_{\text{detritivore}})$$

and $\Delta^{15}N \approx 3.0$ \(\%\) for spiders (Oelbermann and Scheu 2002b).

**RESULTS**

*Prey isotope signatures*

The mixed model analysis revealed that there were no significant differences in the $\delta^{15}N$ values of potential prey organisms between 2006 and 2007 (Table 4.2). There was however, considerable variation in $^{15}N$-enrichment among forest-floor arthropod taxa. Adult flies (Diptera) were significantly enriched in $^{15}N$ relative to primary consumers from the grazing web (herbivores) or from
the detrital web (detritivores and fungivores), regardless of feeding mode (p < 0.0001) (Table 4.3). Fly δ¹⁵N values ranged from 0.01‰ for the mosquito, *Aedes albopictus* (Skuse) (Diptera, Culicidae) to 8.66 ± 0.62‰ for scuttle flies, *Megaselia* spp. (Diptera, Phoridae). This degree of variation among fly taxa is not unexpected, as forest-floor flies exhibit an array of feeding habits including saprophagy, fungivory, predation, and even pollen-feeding (Frouz 1999). Fly δ¹⁵N values were apparently related to resource utilization rather than trophic position, as taxa which scavenge decayed animal matter (e.g. Phoridae, Calliphoridae and Sarcophagidae) or fungal fruiting bodies (Drosophilidae) were more enriched in ¹⁵N than predators (e.g. Dolichopodidae) (Figure 4.1). Excluding flies, detritivorous arthropods, including crickets *Acheta domesticus* (L.) and *Gryllus* spp. (Orthoptera, Gryllidae), wood roaches, *Parcoblatta* spp. (Dictyoptera, Blattellidae), collembolans (Collembola, Entomobryidae), millipedes (Diplopoda, Julida), termites, *Reticulitermes flavipes* (Kollar) (Isoptera, Rhinotermitidae), and litter moth larvae, *Zanclognatha* spp. (Lepidoptera, Noctuidae) exhibited surprisingly little variation in δ¹⁵N values (Figure 4.1). Detritivore δ¹⁵N values ranged from -2.07 to -0.95‰ (δ¹⁵N = -1.26 ± 0.12‰) indicating that despite taxonomic diversity and significant differences in foraging habits, these organisms occupy a similar trophic position in the detrital food web. Only one taxon, the tomocerid collembolans (Collembola, Tomoceridae) were significantly depleted in ¹⁵N relative to other detritivores (δ¹⁵N = -3.76 ± 0.19‰) and appear to fulfill a unique trophic role. With the exception of the tomocerids, detritivores were slightly enriched in ¹⁵N compared with most herbivorous insects, though this pattern was statistically significant only in 2007 (p = 0.002) (Table 4.3). Herbivore δ¹⁵N values ranged from -4.46 to 0.50‰ (δ¹⁵N = -2.46 ± 0.19‰) and there was significant variation evident among taxa with different feeding habits. Most sap-feeding or sucking insects (e.g. planthoppers and aphids) (δ¹⁵N = -3.71 ± 0.31‰) were slightly ¹⁵N-depleted relative to chewing herbivores (e.g. caterpillars and sawfly larvae) (δ¹⁵N = -2.52 ± 0.18‰). Leaf-skeletonizing beetles (weevils and leaf beetles) were the most ¹⁵N-enriched herbivores (δ¹⁵N = 0.39 ± 0.33 ‰) and root-feeding collembolans in the family Sminthuridae were the most ¹⁵N-depleted of organisms analyzed (δ¹⁵N = -6.08 ± 0.16 ‰).

The mixed model analysis indicated that while there was no significant effect of year on arthropod δ¹³C values, there was a significant interaction between prey group and year (p = 0.0009) (Table 4.2). There was no significant annual variation in detritivore δ¹³C values, however herbivores collected in 2006 were slightly enriched in ¹³C (δ¹³C = -28.33 ± 0.52‰) compared with insects collected in 2007 (δ¹³C = -29.16 ± 0.29‰) (p = 0.02) (Table 4.3). Conversely, flies collected in 2006 were significantly depleted in ¹³C (δ¹³C = -25.10 ± 0.28‰) relative to flies collected in 2007 (δ¹³C = -23.88 ± 0.32‰) primarily due to the inclusion of greater numbers of ¹³C-enriched *Drosophila* spp. (Drosophilidae) and flesh flies (Sarcophagidae) in the 2007 samples (Figure 4.1). Detritivorous arthropods, and flies linked to basal
resources in the detrital web, were significantly enriched in $^{13}$C relative to herbivorous insects in both years ($p < 0.0001$) (Table 4.3). Detritivores exhibited a relatively narrow range of $\delta^{13}$C values from -26.56 to -24.19‰ (range = 2.37) further confirming the conclusion that they are generalist omnivores which integrate the range of detrital resources available through polyphagous feeding habits. Conversely, herbivorous insects exhibited much more variation in $\delta^{13}$C values, ranging from -31.86 to -25.00‰ (range = 6.86‰), likely resulting from feeding on wide range of available host plants. Detailed information on species-specific prey $\delta^{13}$C and $\delta^{15}$N values is provided in Appendix A.

**Predator trophic positions ($\delta^{15}$N)**

Spiders were significantly enriched in $^{15}$N relative to herbivores and detritivores (excluding flies) in both 2006 ($\delta^{15}$N spiders = 2.53 ± 0.15‰, $t_{460} = -11.48$, $p < 0.0001$) and 2007 ($\delta^{15}$N spiders = 2.94 ± 0.08‰, $t_{460} = -15.90$, $p < 0.0001$). However, spider $\delta^{15}$N values did not differ significantly from fly $\delta^{15}$N values in 2006 ($t_{460} = 2.02$, $p = 0.05$), and were significantly lower than fly $\delta^{15}$N values in 2007 ($t_{460} = 3.67$, $p = 0.0005$). A continuous spectrum of $\delta^{15}$N values were observed within the spider community, with no evidence for distinct trophic groups (Figure 4.2). Mean spider $\delta^{15}$N values ranged from 0.88‰ for the most $^{15}$N-depleted taxon, the tiny sheetweb weaver *Agyneta* spp. (Linyphiidae) to 4.65‰ for the large, diurnal hunting spider, *Pisaurina mira* (Walckenaer) (Pisauridae). In addition to *P. mira*, the diurnal jumping spider *Maevia inclemens* (Salticidae), the large nocturnal running spider *Gnaphosa fontinalis* Keyserling (Gnaphosidae), the tiny meshweaver *Lathys immaculata* (Chamberlin & Ivie) (Dictynidae) and the orb-weavers *Micrathena gracilis* (Walckenaer) and *M. mitrata* (Hentz) (Araneidae) were also highly $^{15}$N-enriched (Figure 4.3). These species have $\delta^{15}$N values which exceed the baseline $\delta^{15}$N value for detritivorous arthropods ($\delta^{15}$N = -1.26 ± 0.12‰) by more the 5.5‰ indicating that they are nearly two trophic positions above this potential prey group ($\Delta^{15}$N = 3.0‰). At the other end of the spectrum, sheetweb weavers and the dwarf spiders, *Ceratinopsis* spp. (Linyphiidae), and the foliage-running spider *Elaver excepta* (Koch) (Clubionidae) were enriched in $^{15}$N by less then 3.0‰ relative to detritivores and likely feed on more $^{15}$N-depleted prey (Figure 4.3). Other litter-dwelling predators showed similar patterns of $^{15}$N-enrichment. Stone centipedes, *Lithobius* spp. (Lithobiomorpha, Lithobiidae), scolopendomorph centipedes *Theatops* sp. (Scolopendromorpha, Cryptopidae) and earth centipedes (Geophilomorpha, Geophilidae) had $\delta^{15}$N values approximately 3.0‰ greater than detritivores and are thus one trophic position removed from their prey source (Figure 4.3), while *Scolopocryptops* sp. (Scolopendromorpha, Scolopocryptopidae) and the harvestman *Phalangium opilio* (Opiliones, Phalangiidae) were only slightly more $^{15}$N-enriched.

As expected, there was a positive correlation between spider body size (dry mass) and spider $\delta^{15}$N value for cursorial spiders indicating a shift to $^{15}$N-
enriched sprey (e.g. flies or intraguild prey) by larger spiders (Figure 4.4). Adult female body size \( (y = 0.2072x + 0.316, r^2 = 0.29) \) was more strongly related to \( ^{15}\text{N} \)-enrichment than juvenile body size \( (y = 0.0735x + 0.2439, r^2 = 0.04) \), though this may simply be an artifact of the extended range of adult body sizes. With the exception of two large cursorial species, *Anahita punctulata* (Hentz) (Ctenidae) and *Schizocosa* spp. (Lycosidae) adult spiders had similar \( ^{15}\text{N} \) values to juvenile spiders. Adult female *A. punctulata* and *Schizocosa* were enriched by 1.57‰ and 1.37‰ above juveniles, respectively. *Schizocosa* spiderlings collected shortly after dispersal from the mother in the forest retained \( ^{15}\text{N} \) values similar to adult female spiders \( (\delta^{15}\text{N}_{\text{spiderling}} = 2.80 \pm 0.77 \text{‰}) \).

**Predator diets (\( ^{13}\text{C} \))**

Ground-dwelling spiders exhibited a relatively small range of \( ^{13}\text{C} \) values, from \(-26.26\) to \(-24.50 \text{‰} \) (range = 1.76 ‰) compared to the range of carbon signatures observed in available prey species. All spiders were enriched in \( ^{13}\text{C} \) relative to herbivorous insects and spider \( ^{13}\text{C} \) values typically resembled those of detritivorous and fungivorous arthropods and flies (Figure 4.3). Spider dependence on carbon derived from the detrital web was quantified by the IsoError mixing model, which suggested that 70 – 95% of the resources assimilated by litter-dwelling spiders are obtained from the detrital energy channel. The proportional contributions of grazing and detrital prey to the diets of individual species estimated by the model are summarized in Table 4.4. The large sit-and-wait predators, *P. mira* and *Arctosa virgo* (Chamberlin) (Lycosidae) were the most \( ^{13}\text{C} \)-enriched spiders collected from the forest-floor (Figure 4.3), and likely depend almost entirely on detrital prey. Other large sit-and-wait predators, including *Schizocosa* spp. and *A. punctulata* were heavily dependent on detrital resources, with detrital prey comprising at least 90 ‰ of their diets. In contrast the majority of active-pursuit spiders (Gnaphosidae and Corinnidae), as well as the ground crab spiders *Xysticus* spp. (Thomisidae) utilized at least a moderate amount of prey from the grazing subweb. The mixing model indicated that herbivores represented 10 – 25% of assimilated prey for most species. Similar results were obtained for most web-building species, regardless of web style or foraging strategy. The only exceptions were the large, nocturnal retreat web spider, *Wadotes bimucronatus* (Simon) (Amaurobiidae) which were nearly completely dependent on resources from the detrital web (95% detrital prey) and the sheetweb weaver, *Ceratinopsis* spp. (Linyphiidae, Erigoninae) which obtained nearly equivalent proportions of resources from both subwebs. The Isoerror model yielded statistically weak results (i.e. high standard errors, wide confidence intervals) for several species including *Pirata* sp. and *Hogna* sp. (Lycosidae), *Lactroductus* sp. (Theridiidae), *Maevia inclemens* (Salticidae), *Verrucosa arenata* Araneidae and the centipedes, *Scolopocryptops* sp. and Geophilidae spp. (Table 4.4). This is a result of small sample sizes or significant individual-level variation in carbon signatures (i.e. *Lactroductus* sp.), and these species will not be considered.
further in the context of dietary composition. Two centipedes, *Theatops* sp. and *Lithobius* sp., fell outside of the constraints imposed by prey sources. Both were enriched in $^{13}$C ($\delta^{13}$C = -24.26 ± 0.61 ‰ and -24.42 ± 0.57 ‰, respectively) and either feed specifically on detrital prey near the high end of the $^{13}$C spectrum, enriched fungus-feeding prey such as Drosophilidae, or another detrital prey type not included in the study.

**Spider biomass**

The seasonal mean estimate of total spider biomass in the litter layer was 150 mg spider/ m$^2$, which roughly corresponds to direct measurements of spider biomass made in the same forest during the months of August ($\approx$ 110 mg/ m$^2$) and September ($\approx$ 150 mg/ m$^2$) (Chen and Wise 1999). Large cursorial spiders, particularly *Schizocosa* spp., comprised the vast majority of the total forest-floor spider biomass (Figure 4.5). The wolf spider *Schizocosa* spp. alone represented more than 50% of the total spider biomass, while *A. punctulata* and *Arctosa virgo* (Lycosidae) comprised an additional 17%. The other dominant species were nocturnal running species, *Gnaphosa fontinalis* and *Drassyllus* spp. (Gnaphosidae) which together accounted for about 17% of the total spider biomass. The numerically dominant taxon, *Phrurotimpus* spp., represented only 2% of spider total biomass (Figure 4.5). The jumping spiders, *Phidippus* spp. were rarely encountered in the litter layer and represented less than 1% of the total spider biomass on the forest floor, despite being more than 11% of the total number of individuals captured in pitfall traps in 2006 (Chapter 2). All web-building species combined equaled less than 5% of the total forest-floor spider biomass.

**DISCUSSION**

**Prey community**

Terrestrial detrital food webs are typically characterized by high species diversity (e.g. Hunt et al. 1987). Trophic-level omnivory is probably ubiquitous even at the lowest levels of detrital webs, because arthropod ‘primary consumers’ regularly ingest microbial fauna associated with bulk litter or litter fractions (Swift et al. 1979, Polis and Strong 1996, Moore et al. 2004). Previous research using stable nitrogen analysis to examine trophic positions of soil and litter animals has supported this hypothesis with both predators and prey exhibiting continuous gradients of $\delta^{15}$N values (Ponsard and Ardtiti 2000, Scheu and Falca 2000). However, the range of $\delta^{15}$N values observed in detrital predator and prey communities may also be related to variation in $^{15}$N enrichment of heterogeneous basal resources in the detrital food web. Previous researchers have found that there are positive correlations between detrital enrichment in both $^{15}$N and $^{13}$C and decomposition of organic matter, depth in the soil profile and microbial colonization (Nadelhoffler et al. 1988, Ehleringer et al. 2000, Schmidt et al. 2004, Billings and Richter 2006). Organisms that preferentially consume organic matter in late stages of
decomposition, selectively graze saprophytic fungi, or inhabit lower layers of the litter or soil profile are likely to be enriched in $^{15}$N relative to consumers of fresh litter. The current study revealed three relatively distinct trophic groups among the litter-dwelling arthropods; 1) Collembola (Tomoceridae), 2) macroarthropods and Collembola (Entomobryidae) and 3) flies. All of the flies examined were highly enriched in $^{15}$N relative to other arthropods, including predators, and likely feed on organic matter in advanced stages of decomposition.

Most forest-floor macroarthropods, including millipedes, litter moth larvae (Noctuidae, Herminiinae), crickets, wood roaches and subterranean termites were similarly enriched in $^{15}$N. Millipedes and herminiine larvae are rather non-selective consumers of bulk litter and attached microbes (Hohn and Wagner 2000, David and Gillon 2002) and termites consume woody debris, while crickets and roaches are omnivorous and may engage on opportunistic predation and scavenging. The three families of Collembola sampled exhibited highly variable $\delta^{15}$N values. Entomobryidae had nitrogen signatures similar to the macroarthropods. Tomoceridae were significantly depleted in $^{15}$N relative to other microbi-detritivores and Sminthuridae were extremely depleted in $^{15}$N relative to all other animals included in the study. Chahartaghi et al. (2005) proposed three collembolan feeding guilds based on $\delta^{15}$N values: 1) phycophages, which consume lichens, algae and plant tissues (Symphypleona), 2) ‘primary decomposers,’ which consume litter and attached microbes, and 3) secondary decomposers, which selectively graze fungi. Since Sminthuridae were also depleted in $^{13}$C relative to detritivores, it is likely that they are feeding on phycophages and are therefore considered as part of the grazing subweb. The nitrogen signatures observed for Entomobryidae in this system were similar to those of macroarthropods consuming bulk litter (e.g. millipedes) indicating that they likely belong to the ‘primary’ or bulk litter consumer guild. However, Tomoceridae were significantly depleted in $^{15}$N relative to Entomobryidae and other detritivores indicating that they probably preferentially consume resources other than bulk litter. Chahartaghi et al. (2005) placed similarly $^{15}$N-depleted *Tomocerus* spp. in the primary decomposer guild. It is possible that these collembolans selectively consume freshly fallen, non-decomposed litter, while entomobryids consume litter in later stages of decomposition with higher microbial content (Hishi et al. 1997). However, this explanation is unlikely for two reasons. First, tomocerid collembolans have a significantly lower carbon to nitrogen ratio than other detritivore species in this system, including entomobryids (see Chapter 5), indicating that they probably do not feed extensively on nitrogen depleted substrates such as leaf litter. Second, tomocerids were significantly depleted in $^{15}$N relative to herbivorous insects. Since $\delta^{15}$N value of litter increases with increased decomposition and humification (Hyodo et al.2008), detritivores feeding on fresh litter should have $\delta^{15}$N values similar to, or slightly higher than herbivores. The most plausible mechanism explaining low tomocerid $\delta^{15}$N values in this study is that these species are preferentially feeding on
saprophytic litter fungi which may be depleted by more than 3 ‰ relative to the leaf litter substrate (Trudell et al. 2004).

Generalist predator community

The forest-floor spider community likewise exhibited substantial variation in nitrogen signatures, indicating that they likely feed on a wide range of prey items. The most $^{15}$N-depleted web-builders (Linyphiidae) and wandering spiders (Anahita punctulata) were enriched in $\delta^{15}$N by only 2-3 ‰ above values for most detritivores, indicating that they are likely consumers of a combination of primary or secondary decomposers. The most $^{15}$N-enriched wandering spiders, juvenile Pisaurina mira, had $\delta^{15}$N values which exceeded those of detritivores by nearly 6 ‰, indicating that they must feed primarily on $^{15}$N-enriched prey (e.g. flies or other predators). Spiders are exclusively predatory, and many cursorial species are thought to frequently engage in intraguild predation and cannibalism (Wise 1993). Previous researchers have used stable isotope evidence to infer the occurrence of intraguild predation among spiders and other predators (e.g. Sanders and Platner 2007), but the enriched spider $\delta^{15}$N in the detrital web could be related to consumption of $^{15}$N-enriched secondary decomposers as suggested by Scheu and Falca (2000). The current study raises additional questions about the interpretation of predator trophic position based solely on $\delta^{15}$N values, as I observed detritivorous and fungivorous prey species (e.g. fungus gnats, fruit flies) which were equally of more enriched in $^{15}$N than many spider species.

Nearly all previous studies using stable isotope values to examine arthropod food web structure have found that predator values are significantly enriched relative to all potential prey items (e.g. Ponsard and Arditi 2000, Scheu and Falca 2000). In the case of the forest-floor spiders analyzed here, it is possible that flies form a significant component of the diet of some species, including both wandering spiders (e.g. Bardwell and Averill 1997, Ishijima et al. 2006) and web-building spiders (Nentwig 1980, Nentwig 1983, Nyffeler 1999). The combination of $^{15}$N-enriched flies and $^{15}$N-depleted primary or secondary decomposers would likely result in similar spider $\delta^{15}$N values as would intraguild predation. An example of this phenomenon is seen in the foliage-dwelling orb-weavers, Micrathena spp., which were the most $^{15}$N-enriched of all web-building spiders found in the forest-floor food web. Flies are known to constitute a significant proportion of the diets of these spiders (Uetz and Biere 1980), but like other web-builders, it is unlikely that they engage in a significant amount of intraguild predation (Wise 1993).

It has been suggested that the tendency towards intraguild predation is greater among larger spiders as a result of decreased risk associated with attacking smaller predators. Such ontogenetic or size-related shifts in predatory behaviors should result in positive correlations between $\delta^{15}$N value and body size or stage. This hypothesis was only partially confirmed, as there was a positive correlation between the weight of adult female wandering spiders and $\delta^{15}$N value, but little correlation between juvenile $\delta^{15}$N values and
body weight. I hypothesized that spider foraging habit should affect $\delta^{15}N$ values, with active pursuit and stalking predators being the most enriched in $^{15}N$. The large ambush predator, *Pisaurina mira*, exhibited the highest $\delta^{15}N$ values of all spider species in the study. Other large species of active hunters, including *Gnaphosa fontinalis* (Gnaphosidae) and dimorphic jumper, *Maevia inclemens* (Salticidae) had similar $\delta^{15}N$ values. Juveniles of the large burrowing wolf spiders, *Hogna* sp. and *Arctosa virgo* and *Pirata* sp. were also enriched in $^{15}N$ relative to other species despite exhibiting sit-and-wait foraging habits. Conversely, the dominant groups of sit-and-wait predators, the wolf spiders *Schizocosa* spp., and the wandering spider *Anahita punctulata* (Ctenidae) were $^{15}N$-depleted relative to other large species. Surprisingly, the most significantly $^{15}N$-depleted active hunting spiders were sac spiders, *Elaver excepta* (Clubionidae), which are nocturnal hunters, foraging on foliage and in the litter layer. The extremely low $\delta^{15}N$ values associated with this species indicate that they are probably dependent on some combination of microbiodetrivores or tomocerid Collembola and $^{15}N$-depleted herbivorous insects for prey. With the exception of *Micrathena* spp. and the meshweaver *Lathys immaculata* (Dictynidae), web-building spiders were not highly enriched in $^{15}N$. There was no correlation between web spider body size and $\delta^{15}N$ values. Small sheet-web weavers (Linyphiidae) were significantly depleted in $^{15}N$ relative to other spiders, while *L. immaculata* was highly enriched, with nitrogen signatures resembling those of the orb-weaving *Micrathena* spp. *Lathys immaculata* were the smallest spiders included in this study, with adults measuring less one mm in length and weighing approximately half that of *Schizocosa* spiderlings. They build tiny space webs in curled leaves, and may be rather sedentary given the lack of specimens recovered from pitfall traps (Wagner et al. 2003). The most parsimonious explanation for the unusually high $\delta^{15}N$ values observed in this species is that they consume small $^{15}N$-enriched fungus gnats or other Nematocera (e.g. Chironomidae) not included in this study, as do *Dictyna* spp. (Nyffeler 1999). However, it is possible that they engage in cannibalism or prey on tiny predatory mites or spiders not included in this study.

**Spider-mediated links between the grazing and detrital webs**

Carbon source tracing using the IsoError mixing model revealed that strong trophic links exist between ground-dwelling spiders and the detrital food web. Although most spiders were primarily dependent on detrital prey, weak spider-mediated trophic links between the grazing and detrital web were common. The majority of both wandering and web-building species received prey contributions from the grazing subweb representing 10 – 25 % of the total diet. However, several spider species received more substantial prey subsidies in the form of herbivorous insects, representing 30 – 50 % of their diets. The sac spider *Elaver excepta* (Clubionidae) and the harvestman, *Phalangium* sp. (Opiliones: Phalangiidae) incorporated the greatest proportion of herbivorous prey in their diets ($\approx 50\%$). Both species were collected from the understory.
vegetation as well as the litter layer at the study site. While little background information is available for *E. excepta*, most sac spiders are nocturnal hunters which may forage in foliage as well as on the ground (Uetz et al. 1999). *Phalangium* spp. are likewise primarily nocturnal hunters which make daily migrations between the foliage and ground layers (Allard and Yeargan 2005). The diets of the jumping spider, *Phidippus whitmani* (Salticidae) and the ant-mimic *Castianeira cingulata* (Corinnidae) were also composed of more than 30% herbivorous prey. *Phidippus whitmani* is a large, diurnally active running spider which has been observed in low vegetation as well as on the litter surface (*personal observation*), while *C. cingulata* are diurnal running spiders active on the surface of the leaf litter (Uetz et al. 1999).

Conversely, the nursery web spider *Pisaurina mira* (Pisauridae), was enriched in $^{13}$C relative to most other spiders and therefore probably consumes very few herbivorous insects, despite foraging in aboveground vegetation (Carico 1972, Schmitz and Suttle 2001). The mixing model results suggest that this species obtains 96% of its dietary carbon from the detrital subweb in the forest. Additionally, high *P. mira* $\delta^{15}$N values suggest that detrital flies, including $^{13}$C-enriched fruit flies (Drosophilidae) may form an important component of the diet of this species. These conclusions are not in agreement with previous studies which have shown that *P. mira* readily consumes herbivorous insects, such as plant bugs (Hemiptera: Miridae) in cotton fields (Young 1989). However, other researchers have demonstrated that although *P. mira* regularly forages in the upper grass canopy, predation by this species does not significantly affect survival of grasshoppers in an old-field system (Schmitz and Suttle 2001), a finding more consistent with our observations of the isotopic niche of this species. The wolf spider *Arctosa virgo* (Lycosidae) was similarly linked to the detrital web, acquiring about 95% of dietary carbon from detrital sources. *Arctosa* species are nocturnal sit-and-wait predators, which construct silk-lined burrows in the soil (Dondale and Redner 1983). The two dominant taxa of litter-dwelling sit-and-wait predators, *Schizocosa* spp. (Lycosidae) and *Anahita punctulata* (Ctenidae) and the nocturnal running spider, *Drassyllus novus* (Gnaphosidae) likewise received more than 90% of their dietary carbon from the detrital subweb. The remaining wandering species consumed a small, but significant proportion of herbivorous insects ranging from 10–25% of the total diet.

The mixing model suggested that most web-building spider species consume a small proportion of herbivorous insects, ranging from 20–30% of the total diet. However, *Ceratinopsis* spp. were the only web-builders observed to have strong links to the grazing subweb. Erigonid spiders generally construct sheet webs on the ground where they capture high numbers of Collembola, compared to Linyphiinae which construct aerial webs and typically capture higher numbers of herbivores and flies (Harwood et al. 2003, Harwood and Obrzycki 2007). Detritivores, particularly Collembola, are the dominant prey type for erigonids in agroecosystems (Agustí et al. 2003), and other Erigoninae spp. collected from the forest floor in the current study.
seem to adhere to this pattern based on carbon and nitrogen signatures. *Ceratinopsis* spp. however, have been previously documented as inhabitants of aboveground foliage as well as litter (Paquin and Duperre 2006), and were collected in both strata at our study site. Carbon signatures indicated that they consume a significant amount of herbivorous prey, suggesting that they either place webs in low foliage where they are exposed to higher numbers of small herbivores (e.g. leafhoppers, aphids or thrips) or that they selectively consume these organisms when actively foraging in the foliage or litter layer. On the opposite side of the spectrum, the web-builder *Wadotes bimucronatus* (Amaurobiidae) consumed around 95% of prey derived from the detrital subweb. These spiders are large, nocturnal sit-and-wait predators which build retreat webs in the litter layer or under loose bark or stones (Bennett 1987).

**Forest-floor food web structure**

Stable isotope analysis confirmed the hypothesis that the forest-floor detrital food web is characterized by frequent occurrence of omnivory. However this analysis did not completely support the idea that the omnivory leads to lack of defined trophic levels in the detrital food web. This finding is not in agreement with previous studies of the detrital food webs which have documented a continuum of $\delta^{15}N$ values for soil- and litter-dwelling detritivores, which span multiple trophic levels (Scheu and Falca 2000, Ponsard and Arditi 2000). The majority of detritivores included in the current study exhibited a very narrow range of $\delta^{15}N$ values, and therefore appear to occupy a single trophic level. This group likely feeds rather unselectively on litter and associated microbes, though some species, such as crickets, are thought to engage in opportunistic scavenging. The remaining species of non-predatory arthropods fell into two categories, those which were significantly depleted in $^{15}N$ relative to known primary consumers in the grazing subweb, and those which were highly enriched in $^{15}N$. The former group included Tomoceridae (Collembola) and the latter included primarily fungivorous flies. In both cases, these organisms likely represent specialist consumers of fungi (secondary consumers), though the $\delta^{15}N$ values of fungi may be somewhat unpredictable (e.g. Trudell et al. 2004), hence the $\delta^{15}N$ values of some fungivores (e.g. Tomoceridae) may not be enriched relative to lower trophic-level consumers.

This study also provided some evidence for the importance of intraguild predation among forest floor spiders, particularly among large cursorial species including nursery web spiders (Pisauridae), stealthy ground spiders (Gnaphosidae) and jumping spiders (Salticidae) which were highly enriched in $^{15}N$ relative to most available detrital and grazing prey groups. These results agree with previous studies which document the occurrence of intraguild predation among spiders and implicate intraguild predation as an important factor in determining food web structure and dynamics (e.g. Wise and Chen 1999, Rosenheim et al. 2004, Denno et al. 2004). However, the interpretation of trophic position for predators in the forest-floor food web is complicated by the extreme $^{15}N$-enrichment exhibited by many fly species, which resembled
the level of $^{15}$N-enrichment seen in small spiders which may serve as intraguild prey. Therefore, enriched $\delta^{15}$N values in spiders may simply be the result of consumption of fungivorous flies rather than intraguild predation.

Stable isotope analysis also suggested that the forest-floor food web exhibits a highly compartmentalized structure with regards to energy flow between grazing and detrital subwebs (Figure 4.7). Compartments in food web structure are defined as groups of species connected to one another by strong trophic interactions and linked to adjacent compartments by weak interactions (Krause et al. 2003). Habitat heterogeneity, or defined habitat boundaries, such as vertical stratification of litter and vegetation in the forest, can often lead to compartmentalization of energy flow within a food web due to lack of organisms crossing the boundary (Pimm and Lawton 1980, McCann et al. 2005) or prey preference by generalist predators. A study done by Pringle and Fox-Dobbs (2008) used stable isotope analysis to quantify the role of ground-dwelling generalist predators, including spiders, in coupling the canopy and ground-level food webs in a tropical savannah ecosystem. In this case, the authors observed strong trophic interactions between ground predators and herbivorous insects presumably because the herbivores frequently drop to the grass layer. The authors hypothesized that the subsidy of ground-level food webs by falling herbivores may be a ubiquitous feature of forest food webs. The results of the present study seem to refute this hypothesis in the temperate, deciduous forest being studied. Although canopy and understory-dwelling herbivores were frequently found in the leaf litter, stable isotope analysis indicated that they did not comprise a significant proportion of the diet of most dominant spider taxa.

The mixing model provided data on the proportional consumption of carbon originating in the grazing and detrital subwebs by ground-dwelling spiders, but didn’t provide information on interaction strength, or the impact of individual trophic links on food web dynamics. Integrating data on spider biomass with the information on energy flow can allow for an approximate measure of interaction strength. It was difficult to accurately estimate biomass for large active hunting species, particularly jumping spiders or other taxa that may forage both in the foliage and the litter (e.g. *Phidippus whitmani* (Salticidae)), forage primarily at night (e.g. *Elaver excepta*), or simply have the ability to avoid or escape from pitfall traps. The most questionable species in this regard were the jumping spiders, particularly *Phidippus* spp. While juvenile *Phidippus* spp. were abundant in pitfall trap samples (Chapter 2), they may simply be foraging or resting away from the areas where litter was collected for density estimates during the day. Additionally, adult female *Phidippus whitmani*, which were readily collected by hand from the litter surface or lower vegetation during collection of individuals for stable isotope analysis, were rarely found in litter samples or pitfall traps, presumably because their high visual acuity (Jackson and Pollard 1996) allows them to escape easily. Female *P. whitmani* are among the largest species of spiders commonly collected from the litter layer, with a mean body mass of $31.3 \pm 5.4$
Therefore, if only a single female were to be found in every 10 square meters of forest floor habitat, this species would represent more than 2% of the total spider biomass. This discrepancy in adult density estimates may also be common for large web-building spiders, such as *Ariadna bicolor* or *Agelenopsis* spp. where adult females are often found in retreats which may be hidden or positioned in dead wood above the litter surface, or for burrowing wolf spiders, *Hogna aspersa* (Hentz), where burrows are located in the soil.

Wolf spiders (Lycosidae) and wandering spiders (Ctenidae), which account for a large proportion of the biomass of forest-floor spiders, are tightly linked to the detrital subweb (Figure 4.8). The overwhelming dominance of these spiders in the litter layer (70% total spider biomass) suggests that they drive the major proportion of energy transfer between prey and the spider community. Active hunting spiders, such as Gnaphosidae, are weakly linked to the grazing subweb but represent a much smaller proportion of the spider community. Although some numerically abundant groups, particularly the sheetweb and meshweavers (Linyphiidae and Dictynidae), may obtain a significant proportion of their resources from the grazing subweb the total biomass of these spiders is tiny in comparison to the overall forest-floor spider community, and the total amount of grazing subsidies to the detrital web via this route are likely to be minimal. There is one group of spiders which may mediate a strong link between the grazing and detrital subwebs in the forest; the jumping spiders, *Phidippus* spp., as well as *Maevia inclemens* and other species which may exhibit similar trophic habits. This study revealed that *Phidippus whitmani* includes a significant proportion of herbivorous insects as well as detritivores in its diet. This result was not unexpected as many *Phidippus* spp. may preferentially include caterpillars and flies in their diets (reviewed by Jackson and Pollard 1996). Although it was difficult to accurately estimate the biomass of these spiders, *Phidippus* spp. appear to be very common in the litter and understory vegetation in the forest based on pitfall trap captures and visual observations and are the dominant species of Salticidae in the litter layer (Chapter 2). Therefore, herbivore consumption by *Phidippus* may represent a relatively strong trophic link between the grazing and detrital subwebs.

Weak trophic interactions, such as those occurring between most ground-dwelling spiders and aboveground prey in the forest-floor, are important features of food web structure because they can dampen the effects of prey population oscillations on the predator community (McCann et al. 1988, McCann 2000). Teng and McCann (2004) observed that asynchrony in the strength of trophic interactions is the key mechanism leading to stability and species persistence in complex food webs, a concept which also applies to omnivory if it results in unequal energy flow among trophic levels. The results of this study affirm previous assumptions that the grazing and detrital components of the forest food web are distinct subwebs primarily linked at the level of basal resources.

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<td>Titanoecidae</td>
<td><em>Titanoeca americana</em></td>
<td>Titanocid spiders</td>
<td>♂,J</td>
<td>RET</td>
<td>D</td>
<td>L</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.2 Mixed model ANOVA results for the effects of year and functional group (herbivores, detritivores or flies) on prey $\delta^{15}$N and $\delta^{13}$C values

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>$\delta^{15}$N F</th>
<th>P</th>
<th>$\delta^{13}$C F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>1, 152</td>
<td>0.12</td>
<td>0.7279</td>
<td>2.28</td>
<td>0.1332</td>
</tr>
<tr>
<td>Group</td>
<td>2, 152</td>
<td>111.60</td>
<td>&lt;0.0001</td>
<td>101.90</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Year x Group</td>
<td>2, 152</td>
<td>0.82</td>
<td>0.4414</td>
<td>7.34</td>
<td>0.0009</td>
</tr>
</tbody>
</table>
Table 4.3 Mixed ANOVA results for pairwise comparisons between prey groups in 2006 and 2007

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>$\delta^{15}$N</th>
<th>$\delta^{13}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t_{152}$</td>
<td>$P$</td>
</tr>
<tr>
<td><strong>Year effects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbivores</td>
<td>0.65</td>
<td>0.5141</td>
</tr>
<tr>
<td>Detritivores</td>
<td>-0.17</td>
<td>0.8668</td>
</tr>
<tr>
<td>Flies</td>
<td>-1.17</td>
<td>0.2422</td>
</tr>
<tr>
<td><strong>Group effects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbivores vs. detritivores</td>
<td>1.78</td>
<td>0.0772</td>
</tr>
<tr>
<td>Herbivores vs. flies</td>
<td>8.09</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Detritivores vs. flies</td>
<td>-7.21</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>2007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbivores vs. detritivores</td>
<td>3.18</td>
<td>0.0018</td>
</tr>
<tr>
<td>Herbivores vs. flies</td>
<td>11.94</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Detritivores vs. flies</td>
<td>-9.98</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
Table 4.4 Proportional contribution of detrital vs. grazing prey to the diets of litter-dwelling spiders calculated using a single-isotope ($\delta^{13}$C) mixing model

<table>
<thead>
<tr>
<th>Species</th>
<th>Grazing subweb</th>
<th>Detrital subweb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (±S.E.)</td>
<td>95 % CI</td>
</tr>
<tr>
<td><strong>Hunting spiders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anahita punctulata</td>
<td>9.7 ± 5.3</td>
<td>0.0 – 20.2</td>
</tr>
<tr>
<td>Arctosa virgo</td>
<td>4.8 ± 5.9</td>
<td>0.0 – 16.6</td>
</tr>
<tr>
<td>Castianeira cingulata</td>
<td>30.2 ± 8.0</td>
<td>13.4 – 47.0</td>
</tr>
<tr>
<td>Drassyllus aprilinus</td>
<td>12.0 ± 6.9</td>
<td>0.0 – 26.8</td>
</tr>
<tr>
<td>Drassyllus novus</td>
<td>7.9 ± 5.8</td>
<td>0.0 – 19.5</td>
</tr>
<tr>
<td>Elaver excepta</td>
<td>47.9 ± 8.5</td>
<td>27.9 – 68.0</td>
</tr>
<tr>
<td>Gnaphosa fontinalis</td>
<td>17.9 ± 5.3</td>
<td>7.4 – 28.5</td>
</tr>
<tr>
<td>Hogna aspersa</td>
<td>26.6 ± 8.8</td>
<td>0.0 – 54.6</td>
</tr>
<tr>
<td>Maevia inclemens</td>
<td>30.0 ± 12.3</td>
<td>0.0 – 69.0</td>
</tr>
<tr>
<td>Phidippus whitmani</td>
<td>36.6 ± 6.5</td>
<td>23.5 – 49.7</td>
</tr>
<tr>
<td>Phurotimpus spp.</td>
<td>19.4 ± 4.7</td>
<td>10.0 – 28.9</td>
</tr>
<tr>
<td>Pirata sp.</td>
<td>22.0 ± 14.1</td>
<td>0.0 – 82.5</td>
</tr>
<tr>
<td>Pisaurina mira</td>
<td>4.3 ± 6.7</td>
<td>0.0 – 18.0</td>
</tr>
<tr>
<td>Schizocosa spp.</td>
<td>10.0 ± 5.4</td>
<td>0.0 – 20.8</td>
</tr>
<tr>
<td>Xysticus spp.</td>
<td>20.2 ± 7.0</td>
<td>6.1 – 34.3</td>
</tr>
<tr>
<td>Zelotes hentzi</td>
<td>12.2 ± 4.9</td>
<td>2.1 – 22.2</td>
</tr>
<tr>
<td><strong>Web-building spiders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agelenopsis</td>
<td>21.8 ± 7.4</td>
<td>5.8 – 37.7</td>
</tr>
<tr>
<td>Ariadna bicolor</td>
<td>18.5 ± 5.6</td>
<td>7.4 – 29.7</td>
</tr>
<tr>
<td>Ceratinopsis spp.</td>
<td>47.5 ± 6.4</td>
<td>32.8 – 62.1</td>
</tr>
<tr>
<td>Erigoninae spp.</td>
<td>19.6 ± 5.6</td>
<td>7.9 – 31.4</td>
</tr>
<tr>
<td>Lactroductus spp. †</td>
<td>26.5 ± 14.5</td>
<td>0.0 – 72.8</td>
</tr>
<tr>
<td>Lathyss immaculata</td>
<td>24.4 ± 4.9</td>
<td>14.3 – 34.6</td>
</tr>
<tr>
<td>Linphyiinae spp.</td>
<td>26.4 ± 6.0</td>
<td>12.1 – 40.7</td>
</tr>
<tr>
<td>Micrathena spp.</td>
<td>12.0 ± 6.8</td>
<td>0.0 – 27.8</td>
</tr>
<tr>
<td>Titanoea americana</td>
<td>23.9 ± 5.3</td>
<td>13.5 – 34.4</td>
</tr>
<tr>
<td>Verrucosa arenata</td>
<td>13.9 ± 12.0</td>
<td>0.0 – 100</td>
</tr>
<tr>
<td>Wadotes bimucronatus</td>
<td>5.4 ± 7.1</td>
<td>0.0 – 20.1</td>
</tr>
<tr>
<td><strong>Harvestmen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phalangium opilio</td>
<td>47.4 ± 6.2</td>
<td>30.2 – 64.6</td>
</tr>
<tr>
<td><strong>Centipedes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geophilidae</td>
<td>18.9 ± 21.5</td>
<td>0.0 – 87.3</td>
</tr>
<tr>
<td>Lithobius sp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scolopocryptops sp.</td>
<td>15.3 ± 17.4</td>
<td>0.0 – 70.8</td>
</tr>
<tr>
<td>Theatops sp.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† = Outlier removed to prior to fitting model ($\delta^{13}$C = -29.57)
* Consumer $\delta^{13}$C values fell outside the region constrained by the measured prey values resulting in mean proportions of < 0.0% or > 100%
Figure 4.2 $\delta^{15}$N values for litter-dwelling arthropods from the detrital subweb. Primary consumers = eat bulk leaf litter and attached fungi, secondary consumers = eat arthropods and fungi, scavengers = eat decomposed animal and fungal matter (flies).
b) Running & stalking predators

d) Sheet-, space- & orb-web weavers

c) Vagrant web-builders

a) Sit-and-wait & ambush predators

- Detrital prey
- Herbivorous prey
- Spiders (forage in litter)
- Spiders (may forage in vegetation)
- Centipedes (Chilopoda)
- Harvestmen (Opiliones)

\[ \delta^{13}C (\text{‰}) \]

\[ \delta^{15}N (\text{‰}) \]
Figure 4.3 δ^{13}C and δ^{15}N values for litter-dwelling spiders and associated predators by foraging habit. Number samples analyzed given in parentheses.
a) sit-and-wait/ ambush predators: Ctenidae, Ana = Anahita punctulata; Lycosidae, Arc = Arctosa virgo, Hog = Hogna sp., Pta = Pirata sp. Sch = Schizocosa spp.; Phalangiidae (Opiliones), Pha = Phalangium sp.; Pisauridae, Pis = Pisaurina mira (Pisauridae); Thomisidae, Xys = Xysticus spp.; b) running/ stalking predators: Chilopoda, Gph = Geophilidae, Ltb = Lithobius spp., Scl = Scolopocryptops sp., Thp = Theatops sp.; Clubionidae, Ela = Elaver excepta; Corinnidae, Cas = Castianeira cingulata, Phr = Phrurotimpus spp.; Gnaphosidae, Dap = Drassyllus aprilinus, Dnv = D. novus, Gnp = Gnaphosa fontinalis, Zel = Zelotes hentzi; Salticidae, Mav = Maevia inclemens, Phd = Phidippus whitmani, c) vagrant web-builders: Agelenidae, Age = Agelenopsis spp.; Amaurobiidae, Wad = Wadotes bimucronatus; Segestriidae, Ari = Ariadna bicolor; Titanoeicidae, Tna = Titanoeca americana; and d) sheetweb, sheet-, space- and orb-web weavers: Araneidae, Mgc = Micrathena gracilis, Mmt = M. mitrata, Ver = Verrucosa arenata; Linyphiidae, Crt = Ceratinopsis spp., Eri = Erigoninae spp., Lin = Linyphiinae spp.; Theridiidae, Lac = Lactrodectus sp.
Figure 4.4 Relationships between individual spider body weights and stable isotope signatures for a) $\delta^{15}$N and b) $\delta^{13}$C
Figure 4.5 Comparisons between spider density (individuals/ m$^2$) in the litter layers and biomass of dominant spider taxa (mg/ m$^2$).
Figure 4.6 Predator-mediated energy flow between the grazing and detrital subwebs. Arrows widths represent the strength of the trophic link between spiders and resources from either web estimated as the proportion of dietary carbon obtained from grazing versus detrital prey. Ovals represent spider taxa, single outline = cursorial spiders, double outline = web-builders. Actual estimates of dietary contribution of grazing and detrital prey to spiders are provided as percentages in the figure.
Figure 4.7 Strength of trophic links between spiders and grazing versus detrital prey. Arrow direction indicates estimated energy flow and width represents relative interaction strength. Circle size represents proportion of total spider biomass represented by spider feeding guilds. Spider feeding guilds are 1) sit-and-wait spiders (Lycosidae, Ctenidae and Pisauridae); 2) active hunters (Gnaphosidae, Corinnidae); 3) stalking spiders (Salticidae); 4) vagrant web-builders (Segestriidae, Titanoecidae, Amaurobiidae, Agelenidae); 5) Ambush predators (Thomisidae); and 6) sheetweb or meshweavers (Linyphiidae, Dictynidae).
CHAPTER V

Modeling resource utilization by forest-floor spiders using stable isotopes ($\delta^{13}$C and $\delta^{15}$N) and carbon-nitrogen stoichiometry

SUMMARY

Wolf spiders (Araneae, Lycosidae) and their relatives are among the most abundant predatory arthropods in the leaf-litter food web of the temperate deciduous forest. These spiders are highly polyphagous and prey selection may be related to availability of prey in the environment, ease of capture, or risk associated with predation. Optimal foraging by spiders may also drive resource utilization as spiders attempt to maximize prey quality and minimize nutritional deficiencies. Previous research has suggested that collembolans, particularly large litter-dwelling Tomoceridae or Entomobryidae, are important prey in the diets of forest-floor wolf spiders, though spiders exhibit increased fitness and survival rates when provided with diets consisting of mixed prey types. The objectives of this study were to model the contributions of available prey groups to the diets of two forest-floor spiders, the wolf spider Schizocosa spp. and the wandering spider, Anahita punctulata (Hentz) (Araneae, Ctenidae) using stable carbon and nitrogen isotope ratios ($\delta^{13}$C and $\delta^{15}$N), and to examine stoichiometric constraints associated with spider predation on forest-floor arthropods. The stable isotope signatures of numerous potential prey types were combined a priori into seven statistically distinct source values and input into a multi-source, dual-isotope mixing model, IsoSource. The IsoSource model clearly implicated one of the prey groups, comprised of a single family of collembolans (Tomoceridae, Collembola), as a key resource for the juvenile stages of both spider species. Analysis of C:N stoichiometric ratios suggested that spiders should experience nitrogen-limited growth on diets of all detrital prey types with the exception of Tomoceridae and intraguild prey. The IsoSource model results support stoichiometric theory in supporting a strong trophic link between Tomoceridae and both spider species in the forest-floor food web.
INTRODUCTION

Cursorial spiders are a ubiquitous and abundant group of generalist predators in terrestrial food webs. Natural ecosystems, such as unmanaged forests or grasslands often support a diverse assemblage of ground-dwelling cursorial spiders, which are tightly linked to prey resources from the detrital subweb. The mechanisms sustaining this diversity and permitting coexistence within spider communities are not well understood, nor are the effects of predator diversity on prey populations. Research suggests that spiders are typically subject to resource limitation in nature (reviewed by Wise 1993), an assumption which is supported by the fact that supplementation of detrital resources often leads to increased densities or biomass of ground-dwelling species in a range of ecosystems (e.g. Chen and Wise 1999, Marshall et al. 2000, Halaj and Wise 2002, Wise et al. 2006). However, experimental manipulation of spider densities has also demonstrated the occurrence of top-down limitation of prey populations by forest-floor wolf spiders (Buddle 2002, Wise 2004). Forest-floor spider species are likely to have overlapping habitat domains and dietary composition related to foraging habits and body size (Nyffeler 1999). Local suppression of important detrital prey groups, such as Collembola, by wolf spiders may have significant effects on other forest-floor spider populations and may ultimately have a significant impact on spider community composition. Competition for shared prey is not likely to be an important force in structuring communities of web-building spiders (Wise 1993, Marshall and Rypstra 1999), but there is little empirical evidence to support or deny the occurrence of exploitative competition among cursorial species (but see Wise and Wagner 1992). Resource partitioning may arise as a mechanism by which coexisting spiders avoid or decrease competitive interactions including intraguild predation, and may often involve utilization of alternative, often low quality resources by either species. Additionally, ontogenetic shifts in resource or habitat utilization by spiders may decrease intra-specific competition and cannibalism. This study documented species-specific differences and ontogenetic shifts in dietary composition for two common litter-dwelling cursorial spider taxa, wolf spider in the genus *Schizocosa* (*S. ocreata* (Hentz), *S. saltatrix* (Hentz), *S. stridulans* Stratton) (Araneae, Lycosidae) and the wandering spider *Anahita punctulata* (Hentz) (Araneae, Ctenidae) in a deciduous forest.

Cursorial spiders often exhibit a range of foraging habits, associated with varying degrees of polyphagy or diet breadth (Nyffeler 1999). While some species may be true generalists in the sense that they consume a broad range of prey with dietary composition based on prey availability in the environment, most species probably exhibit some degree of prey selectivity (Nentwig 1986, Nyffeler et al. 1994). Dominant prey groups available for spiders in the forest-floor habitat include detritivorous microarthropods such as Collembola and flies (Diptera), macroarthropods such as crickets (Gryllidae, Orthoptera), roaches (Blattodea) and millipedes (Diplopoda) as well as other predators (Chapter 2). Laboratory research suggests potential prey varies in quality, palatability and possibly toxicity to spiders (Toft and Wise 1999a). Previous authors have suggested that
spiders select high quality prey types, or prey which optimizes consumption of amino acids, nutrients, protein or lipids (Greenstone 1979, Mayntz et al. 2005). Laboratory studies also suggest that spiders can select optimal prey types necessary to correct nutrient deficiencies (Mayntz et al. 2005). Since spiders exhibit higher rates of survival, growth and fecundity on mixed diets even when compared to diets of the highest quality single prey type (Toft and Wise 1999, Oelbermann and Scheu 2002a), it seems unlikely that any single prey group is sufficient to completely fulfill the spider’s nutritional requirements.

The present study utilized stable isotope ratios ($\delta^{13}$C and $\delta^{15}$N) to assess the contribution of specific types of available detrital prey to the diets of Schizocosa spp. and Anahita punctulata. Dietary contributions were derived using IsoSource, a multi-source dual isotope mixing model (Phillips and Gregg 2003) for both adult females and juveniles of each species. I tested the general hypothesis that species- and stage-specific differences in resource utilization and trophic position exist between these two species. I assessed ontogenetic differences in resource utilization, since adult females are expected to exhibit prey switching behaviors in response to increased predator to prey body size ratios, need for additional nutrients for reproduction, or increased ability to engage in intraguild predation. In addition I examined the nutritional value of potential prey groups to ascertain whether highly nutritious, nitrogen-rich prey (e.g. intraguild prey) represented a greater proportion of the spiders’ diets than nitrogen-depleted prey.

**MATERIALS AND METHODS**

**Sample collection and stable isotope analysis**

Stable isotope analysis of Schizocosa and A. punctulata, forest-dwelling Diptera and alternate prey were conducted as part of a larger scale study on the forest-floor spider community (Chapter 4). Spiders and prey were hand-collected from the forest floor from May through September of 2007, and collection and sample storage followed the protocols outlined above for spiders. Prior to analysis, spiders and prey were dried in a 60°C oven for 24 – 48 hrs and ground to a fine powder using a micropestle. Approximately 1 mg of dried animal tissue was required to obtain measurable quantities of nitrogen using isotope ratio mass spectrometry. Therefore multiple individuals of small organisms (e.g. spiderlings and many prey species) were combined to form a single sample, while larger spiders were homogenized and a 1-2 mg subsample collected for analysis. All samples were packed into 5 x 9 mm tin capsules (Costech Analytical Technologies Inc., Valencia, CA, USA). Stable isotope ratios ($\delta^{13}$C and $\delta^{15}$N) and atomic %C and %N were measured using a PDZ-Europa elemental analyzer coupled to a PDZ-Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Analyses were done by the Stable Isotope Research Facility at the University of California-Davis. Stable isotope values are expressed in $\delta$ units or the isotopic ratio relative to a known standard using the equation:
\[ \delta X = \left( \frac{R_{\text{sample}}}{R_{\text{std}}} - 1 \right) \times 1000 \]

where \( \delta X (\%o) = \delta^{13}C \) or \( \delta^{15}N \), \( R^{13}C/^{12}C \) or \( R^{15}N/^{14}N \) and the standards are Pee Dee Belemnite for \( \delta^{13}C \) and atmospheric nitrogen for \( \delta^{15}N \).

**Predator-prey stoichiometry**

A stoichiometric approach was used to compare the potential value of each prey type to *Schizocosa* and *A. punctulata*. Since organismal C:N ratio is under tight homeostatic regulation, discrepancies between spider and prey C:N ratios are expected to result in nitrogen limitation for spiders feeding on prey with high C:N ratios (Fagan and Denno 2004). The minimum value of prey C:N ratio which should result in nitrogen limitation and associated decreased growth and fitness potential for the spider can be estimated using the threshold elemental ratio (Urabe and Watanabe 1992, Sterner and Elser 2002). Threshold elemental ratios (TER\(_{C:N}\)) were calculated for *Schizocosa* and *A. punctulata* using the formula

\[ \text{TER}_{C:N} = \frac{\alpha_N}{\alpha_C} \cdot C : N_{\text{spider}} \]

where \( \alpha_N \) is the maximum gross growth efficiency for nitrogen, \( \alpha_C \) is the maximum gross growth efficiency for carbon and \( C : N_{\text{spider}} \) is the C:N ratio of the spider. There is a dearth of published data regarding the efficiency of carbon or nitrogen assimilation by spiders or other arthropods, so following the strategy of previous authors (e.g. Matsumura et al. 2005), I have used values of \( \alpha_N = 0.70 \) and \( \alpha_C = 0.65 \) to indicate that spiders are probably not able to extract and assimilate one hundred percent of available carbon and nitrogen found in prey, but should be slightly more efficient at extracting nitrogen than carbon due to its relative scarcity in nature (Fagan et al. 2002, Fagan and Denno 2004, Matsumura et al. 2005). By rearranging the equation, spiders should be nitrogen-limited when

\[ \frac{C : N_{\text{prey}}}{C : N_{\text{spider}}} > \frac{\alpha_N}{\alpha_C} = \frac{C : N_{\text{prey}}}{C : N_{\text{spider}}} > 1.07 \]

**Spider dietary composition**

Spider dietary composition was modeled using a multi-source, dual-isotope mixing model, IsoSource (Phillips and Gregg 2003). The IsoSource model was selected because the number of potential prey sources available to litter-dwelling spiders precludes calculation of a unique solution to a simple linear mixing model with two isotopes. The model returns frequency distributions for proportional source contributions resulting from all feasible solutions to the mass balance equations within an acceptable tolerance. The source increment used for this analysis was 1% and the mass balance tolerance was set to 0.1%. A primary
assumption of the model is that all sources are significantly different from one another in isotopic space and the total number of sources can not be greater than ten (Phillips and Gregg 2003). The isotopic signatures obtained for *Schizocosa* and *A. punctulata* were corrected for trophic fractionation using estimated trophic-level enrichment values derived for cursorial spiders of $\Delta^{15}N = 3.0$ and $\Delta^{13}C = 0.4$ (Oelbermann and Scheu 2002b). Dietary composition was modeled separately for adult female and juvenile stages of each species.

Differences among prey species ($\delta^{13}C$ and $\delta^{15}N$) were assessed using non-parametric (permutation-based) multivariate analysis of variance (NPMANOVA), with multiple comparisons corrected using the false discovery rate (FDR) method (Benjamini and Hochberg 1995). Potential prey sources used in the model were isolated from a data set containing data for a wide array of potential prey items from the forest-floor food web, such as detritivorous and herbivorous arthropods, as well as Diptera (chapter 2). Potential prey items for each species and stage of spider were included based on predator to prey body size ratio. Extremely small prey types, weighing less then 1% of the total mean body weight of the spiders, were excluded from the models for adult females (Moulder and Riechle 1972). Several other prey types which may be toxic, unpalatable, or infrequently encountered by *Schizocosa* or *A. punctulata* were excluded, including millipedes, large Diptera and litter moth larvae, herbivorous flies and termites (e.g. Moulder and Reichle 1972). Intraguild prey was included in the model, and represented by small spiders. The values used for IG prey were derived from the spider isotope data presented in Chapter 3, by calculating the mean value of all spiders sampled which fell into the smallest size class, weighing less than 1 mg. This value included samples of a number of small spider species, including sheetweb weavers (Linyphiidae, Linyphiinae), dwarf weavers (Linyphiidae, Erigoninae) and *Phrurotimpus* spp. (Corrinnidae), as well as juveniles of larger species including the web-building *Agelenopsis* spp. (Agelenidae), *Titanoeca americana* (Titanoecidae), *Ariadna bicolor* (Segestriidae) and the ambush predator, *Xysticus* spp. (Thomisidae). *Schizocosa* spiderlings collected after they had dropped from the female were also included in this value. Prey species represented by a single value (n=1) were excluded from the model because the NPMANOVA procedure requires that all variables be replicated. Non-significantly different species were pooled and used as endpoints for the IsoSource mixing model. Statistical analyses were done using the PAST (PAleontological STatistics) software package (Hammer et al. 2001).

**RESULTS**

**Predator-prey stoichiometry**

The threshold element ratios ($TER_{C:N}$) were $4.31 \pm 0.08$ for *Schizocosa* and $4.23 \pm 0.08$ for *A. punctulata*. Therefore, *Schizocosa* and *A. punctulata* should experience nitrogen-limited growth on diets consisting of any prey with a higher C:N ratio than 4.31 and 4.23, respectively. In this system, *Schizocosa* and *A. punctulata* should experience some level of nitrogen deficiency when consuming all prey types other than Tomoceridae (Collembola) or when preying upon each
other. Surprisingly, tomocerid Collembola had a lower C:N ratio (3.80 ± 0.09) and slightly higher body nitrogen content (13.43 ± 0.38%) than either *Schizocosa* (C:N = 4.00 ± 0.07, %N = 12.66 ± 0.22) or *A. punctulata* (C:N = 3.93 ± 0.07, %N = 13.17 ± 0.23). This indicates that spiders exclusively consuming tomocerids should have an excess of nitrogen in their diets (C:N<sub>prey</sub> / C:N<sub>spider</sub> < 1.00). Mutual predation, or *Schizocosa* consumption of *A. punctulata* and vice versa, would also result in avoidance of nitrogen limitation for both species (C:N<sub>prey</sub> / C:N<sub>spider</sub> ≈ 1.00). All other potential prey groups in the survey, including small spiders which represented intraguild prey, had higher C:N ratios than *Schizocosa* or *A. punctulata* which should result in nitrogen limitation for both species (C:N<sub>prey</sub> / C:N<sub>spider</sub> > 1.07) (Figure 5.1). Abundant detritivores, such as wood roaches (C:N = 4.47 ± 0.10) and crickets (C:N = 4.57 ± 0.13), exhibited only minor stoichiometric imbalances with *Schizocosa* and *A. punctulata*. However, some of the most abundant litter-dwelling flies in the forest, Drosophilidae (C:N = 4.88 ± 0.17) and fungus gnats (Sciaridae) (C:N = 4.92 ± 0.13) lead to moderate nitrogen deficiency (C:N<sub>prey</sub> / C:N<sub>spider</sub> > 1.20) and millipedes which have extremely low nitrogen content and high C:N ratios (%N = 4.53 ± 0.26, C:N = 7.27 ± 0.24) are extremely poor quality prey for spiders. Exclusive or frequent consumption of millipedes would likely lead to severe nitrogen limitation for both spider species (C:N<sub>prey</sub> / C:N<sub>spider</sub> = 1.85).

**Arthropod stable isotope ratios**

Flies were universally enriched in $^{15}$N relative to detritivores and herbivores collected from the forest floor (Figure 5.2). Complete details regarding detritivore, herbivore and spider stable isotope ratios can be found in Chapter 3 and Appendix A. Flies exhibited an extremely broad range of $\delta^{15}$N values, from -2.58 – 8.66 ‰ (range = 11.24 ‰) indicating the presence of at least three trophic levels assuming $\Delta^{15}$N ≈ 3.4 ‰ (Minagawa and Wada 1984, De Niro and Epstein 1981). The Diptera collected from the forest floor and understory layers were predominantly detritivores or microbivores, though scavengers, predators and parasitoids were also found. Most of the flies had $\delta^{13}$C values similar to those of detritivorous arthropods collected from the litter layer ($\delta^{13}$C<sub>detritivore</sub> = -25.52 ± -0.17). A few fly taxa were significantly depleted in $^{13}$C and had carbon signatures similar to herbivorous insects ($\delta^{13}$C<sub>herbivore</sub> = -28.80 ± 0.26) (Chapter 2), including adult tachinid flies (Tachinidae) and blow flies, *Calliphora* sp. (Calliphoridae). Pomace flies (Drosophilidae) and one species of flesh fly (Sarcophagidae) were enriched in $^{13}$C relative to other flies and detritivores (Figure 5.2).

A total of seven statistically distinct prey clusters were identified using the NP-MANOVA procedure with multiple comparisons and subsequent grouping of similar groups. Stable isotope values ($\delta^{15}$N and $\delta^{13}$C) for detrital and intraguild prey presented in chapter 2, as well as values for fly taxa discussed here, were included in the analysis. The seven clusters included three groups of Diptera, herbivorous insects, two groups of micro-detritivores and a group composed of fungus gnats (Mycetophilidae and Sciaridae) and intraguild prey (Araneae) (Figure 5.2). In all cases except for the cluster containing fungus gnats and spiders, there was either a taxonomic or functional (resource utilization)
relationship between all species in the group based on prior knowledge about feeding habits. Diptera fell into three statistically distinct clusters: the first cluster encompassed all species of pomace flies (Drosophilidae spp.) and one species of flesh fly (Sarcophagidae sp.), the second comprised a second flesh fly, the blow fly *Lucilia* sp. (Calliphoridae), wood gnats (Anisopodidae spp.) and long-legged flies (Dolichopodidae sp.), and the final cluster contained only the scuttle flies, *Megaselia* spp. (Phoridae). All three groups were significantly enriched in $^{15}$N compared to other organisms (including spiders), and the fruit fly group was slightly enriched in $^{13}$C relative to all other predators and prey analyzed (Figure 5.2). The herbivore group included a variety of herbivorous insects potentially found on the forest floor (see Chapter 3). The microbi-detritivores formed two groups: 1) Tomoceridae (Collembola) and 2) wood roaches (*Parcoblatta* spp., Blattellidae), crickets (*Acheta domesticus* and *Gryllus* sp.) and Entomobryidae (Collembola). The fungus gnats (Sciaridae and Mycetophilidae) were statistically indistinguishable from the small spiders (IG prey) in this analysis. Therefore, IG prey and fungus gnats were treated as a single source value in the juvenile model, and mechanisms for interpreting the potential contributions of each group to the spiders’ diets were applied during the analysis.

Both *Schizocosa* ($\delta^{13}C = -24.98 \pm 0.10$) and *A. punctulata* ($\delta^{13}C = -25.03 \pm 0.09$) had $\delta^{13}C$ values which closely resembled $\delta^{13}C$ values obtained for detrital prey ($\delta^{13}C = -25.52 \pm 0.14$) and thus probably obtain the majority of their resources from the detrital food web (Chapter 3). However, *Schizocosa* ($\delta^{15}N = 2.33 \pm 0.19$) were overall slightly enriched in $^{15}$N over *A. punctulata* ($\delta^{15}N = 1.82 \pm 0.20$), and both species exhibited significant ontogenetic shifts in $\delta^{15}N$ values, with adult females enriched in $^{15}$N relative to juveniles (Figure 5.3). There was no correlation between individual female *Schizocosa* body mass and $^{15}$N-enrichment ($r^2 = 0.05$), and there was a very weak negative correlation between juvenile body mass and $\delta^{15}N$ values ($r^2 = 0.13$) (Figure 5.3a). Conversely there was a weak positive correlation between female *A. punctulata* body mass and $\delta^{15}N$ values ($r^2 = 0.12$), but no correlation between juvenile body mass and $^{15}$N-enrichment ($r^2 = 0.01$) (Figure 5.3b).

**IsoSource mixing model**

The six prey groups identified by the NPMANOVA were used as sources in the multi-source mixing model, IsoSource. The model was run separately for adult females and juveniles of each species (Figure 5.4). Adult female spiders are relatively large compared to many of the prey items analyzed, and analysis of predator-prey body size ratios resulted in exclusion of several potential prey taxa deemed too small (prey mass < 1% of mean female spider body mass) to be regularly consumed by adult spiders. This resulted in exclusion of fungus gnats (Diptera, Sciaridae) (individual mass = 0.12 ± 0.02 mg) and scuttle flies (Diptera, Phoridae) (individual mass = 0.14 ± 0.02) from the adult models. As Phoridae exhibited significantly different stable isotope signatures from other potential prey (Figure 5.2), removal of this taxon led to the use of only five sources in the adult mixing models. Exclusion of fungus gnats and entomobryids did not significantly alter the mean source values for their respective prey clusters, but does aid in
the interpretability of the model as both groups now represent taxonomically distinct groups; intraguild prey (small spiders) and omnivorous crickets (*Gryllus* sp. and *Acheta domesticus*) and roaches, respectively. The mixing polygon diagrams for the juvenile and adult models are presented in Figure 5.4.

The IsoSource mixing model provided ambiguous results for the contributions of most prey groups to the diets of adult *Schizocosa* and *A. punctulata*, but gave slightly better resolution for juveniles. As there are no unique solutions available for multi-source mixing models, the mean values provided in Table 5.1 serve only as an estimate of the potential contribution of each resource to the spiders’ diets. The range of feasible solutions calculated by the IsoSource program is a better indicator of the potential contribution of each resource. In the case of current model, the range of feasible contributions for each group is bounded by zero at the low end (Table 5.1). This indicates that there is no evidence for definitive inclusion of any prey group, with the exception of tomocerid Collembola, in the diet of either spider species. However, the shape of the frequency distributions varies significantly between prey groups and spiders, and can still provide general information regarding the importance of a given resource in a spider’s diet (Figures 5.5 – 5.8). The results of the mixing model do confirm that Diptera are unlikely to be a large component of the diet of juvenile *Schizocosa* or *A. punctulata* (Figure 5.5, 5.6). Scuttle flies (Phoridae), in particular, appear unlikely to serve as a resource for juveniles of either spider species, despite their abundance in the litter layer. However, it does seem likely that fruit flies (Drosophilidae) or other small 13C-enriched flesh flies (Sarcophagidae) form a small but definite component of the diet of juvenile *Schizocosa* (Figure 5.5) and may account for some of the infrequent occurrences of fly predation observed in juveniles of this species. However, the higher rates of fly predation by adult *A. punctulata* did not translate into species-specific differences in the results of the stable isotope model for *A. punctulata* compared to *Schizocosa* (Figure 5.7, 5.8). Both species are likely to incorporate more flies into their diets as adults than as juveniles though the dietary composition of the species did not differ significantly in any regard, as indicated by their isotopic similarity.

Other prey types included in the mixing model analysis included herbivores, detritivores and intraguild prey. Herbivorous insects contribute little to the diet of juvenile spiders of either species (5.5, 5.6), but may be a small component of the diet of adults (Figure 5.7, 5.8). This is consistent with the results of the analysis of carbon utilization from the grazing versus detrital subwebs by forest-floor spiders described in Chapter 3. The mixing model was unable to provide any definitive information regarding the contributions of most detritivores or intraguild prey to the diets of spiders. This is largely a result of the location of these two data points relative to the other source values. Since they are inside the mixing polygon, the model is unable to obtain a constrained solution for these sources without external information (Phillips and Gregg 2003). The high upper bounds of the range of feasible contributions calculated for detritivores (71-77%) indicates that crickets and roaches could potentially be a major component of the diet of adult spiders. The mixing model did provide
strong evidence for the importance of tomocerid Collembola in the diet of juvenile spiders, particularly juvenile *A. punctulata* (feasible range = 26 – 68% total diet) (Figure 5.6). Conversely, the contribution of Tomoceridae to the diets of adult spiders appears to be considerably less, and is not well-defined by the model.

**DISCUSSION**

*Prey quality and optimal foraging*

Optimization of foraging strategies can have a significant influence on spider fitness (e.g. Toft 1999). Prey selection by spiders may be related to prey availability, predator-prey body size ratio, prey quality or nutritional value (Greenstone 1979, Mayntz et al. 2005), and risk associated with prey capture (Walker and Rypstra 2003). Previous research has suggested that stoichiometric imbalances in predator-prey C:N ratios and associated nitrogen deficiencies can lead to nitrogen-limited growth in generalist predators such as spiders (Fagan and Denno 2004). In this study I found that one particular group of prey, tomocerid Collembola which is often implicated as an important resource for litter-dwelling spiders, had significantly higher body nitrogen content and lower C:N ratio than all other prey species analyzed. Additionally, tomocerids exhibited lower C:N ratios than *Schizocosa, A. punctulata* or small spiders considered as potential intraguild prey. This pattern is the opposite of the stoichiometric imbalance typically observed between arthropod predators and herbivorous insect prey, and as suggested by Fagan et al. (2002), may result from nitrogen immobilization by Collembola (see Hopkin 1997). This observation suggests that tomocerids should be an extremely high value resource for litter-dwelling spiders in general, as a diet consisting entirely of Tomoceridae would result in an excess of nitrogen for the spiders, and confirms the results of laboratory studies which suggest that spiders consuming diets containing tomocerids experience increased growth and survival relative to other prey types including flies (Toft and Wise 1999). The threshold elemental ratio, developed by Sterner and Elser (2002) suggests that both spider species should incur growth penalties when feeding on all prey types other than tomocerids, small spiders and other intraguild prey, or engaging in mutual predation.

The assumption of nitrogen limitation in spiders does not take into account the potential for restrictions to growth and fitness imposed by other limiting factors, such as phosphorus or nutrients which were not measured in this study (e.g. Sterner and Elser 2002, Mayntz and Toft 2001, Mayntz et al. 2005). Previous researchers have found that wolf spiders generally experience higher fitness in laboratory assays when provided with a diet containing a mixture of non-toxic species than when reared on single-species diets (Uetz et al. 1992, Toft and Wise 1999a, Oelbermann and Scheu 2002a). Toft and Wise (1999) found that survival of juvenile *Schizocosa* reared on a diet consisting exclusively of tomocerid Collembola was similar to that of individuals consuming a mixture of tomocerids and fruit flies (*Drosophila melanogaster*), but that growth rates for individuals reared on the mixed diet were significantly higher indicating that the flies contributed something which may be lacking in the tomocerids. The authors
also observed significantly lower growth rates and decreased survival in *Schizocosa* reared on fruit flies alone. The necessity for inclusion of diverse prey types in the diet of wolf spiders and the apparent nutritional benefits of flies are supported by the results of the IsoSource model which indicate likely utilization of multiple prey groups, and are in agreement with the finding of consistent occurrence of Diptera consumption by these spiders in the forest documented in Chapter 4 of this dissertation.

**Resource utilization by forest-floor spiders: implications for food web dynamics**

The mixing model analysis used in this study was only able to provide a rudimentary assessment of the contributions of most prey groups to the diets of forest-floor spiders. The one exception was tomocerid collembolans, which were directly implicated as a key resource, particularly for juvenile spiders. This observation confirms previous studies which have inferred the central role of collembolans in the diet of the wolf spiders based on Collembola responses to alterations in spider densities (Wise and Wagner 1992, Buddle 2002, Wise 2004), and supports the idea that collembolans are more likely to be important prey for smaller stages of large wolf spider species than for adults (Sanders and Platner 2007, Oelbermann et al. 2008). Although there is probably a strong overlap in resource utilization by the juvenile stage of these two species, *Schizocosa* may have a slightly broader dietary range than does *A. punctulata*. The benefits conferred on *Schizocosa* by greater dietary breadth may include the ability to utilize alternative resources, such as fruit flies, during periods of scarcity of preferred resources, in this case tomocerid collembolans.

The mixing model analysis uncovered significant differences in resource utilization between adults and juveniles of both species. Ontogenetic shifts in spider diets were exemplified by the significant increases in $\delta^{15}N$ values in female spiders which imply inclusion of higher proportions of $^{15}N$-rich prey in the adult diet. In most cases $\delta^{15}N$ values increase predictably with increased trophic position, so that consumers are enriched in $^{15}N$ relative to their resources (DeNiro and Epstein 1981, Minagawa and Wada 1984, McCutchan et al. 2003). Therefore, increased predator $\delta^{15}N$ values could provide evidence for the occurrence of intraguild predation. However, in the leaf-litter food web, I observed that most flies have highly enriched $\delta^{15}N$ values which are not necessarily correlated with trophic position, but may be related to other factors such as variation in resource $\delta^{15}N$ values or taxon-specific differences in nitrogen fractionation (Vanderklift and Ponsard 2003). The abundance of $^{15}N$-enriched prey in the forest ecosystem makes interpretation of female spider trophic position difficult, as high $\delta^{15}N$ values could result from either intraguild predation or consumption of flies. However, gut content analysis confirmed that female *A. punctulata* consumed flies significantly more often than female *Schizocosa* or juveniles of either species (Chapter 3). Therefore ontogenetic increases in *A. punctulata* $\delta^{15}N$ values may be due to increased predation on Diptera. Similar $^{15}N$-enrichment in adult *Schizocosa* compared to juveniles were not due to
changes in Diptera consumption (Chapter 3) indicating a developmental shift towards increased intraguild predation by this species.

**Conclusions**

The stable isotope analysis employed in this study revealed a striking degree of similarity in resource utilization between two co-existing forest-floor spiders. While there is no empirical evidence documenting competition for shared resources between these spiders, the significant overlap in resource utilization suggests that under conditions of resource limitation such interactions may occur. The IsoSource model also provided definitive insights into the importance of two major forest-floor arthropod taxa to the diets of these spiders, tomocerid collembolans and flies. The finding that tomocerids are a key resource for *Schizocosa* spp. and *A. punctulata* lends support to the results of previous studies, such as Wise (2004), which have postulated the importance of a tomocerid-spider pathway of energy flow within the forest-floor food web. Previous conclusions regarding the importance of this trophic link have been based on the effects of experimental manipulations of spider densities on prey populations, which suggest that forest-floor spiders, especially *Schizocosa* spp., may actively limit tomocerid densities in the litter layer (Lawrence and Wise 2004, Wise 2004). The current study was the first to uncover direct evidence of a strong and persistent trophic interaction between cursorial spiders and tomocerid collembolans in the forest. Interestingly, this result was in agreement with, and supported by the theory of carbon-nitrogen stoichiometry. The unusually low C:N ratio observed for Tomoceridae make this taxon an extremely high value prey for spiders supposing that nitrogen is a limiting factor in the forest-floor food web. The IsoSource model suggested that Tomoceridae formed a significantly greater proportion of the diet of these spiders than did any other potential prey type, especially flies. Overall, the model indicated that flies are likely to form a negligible component of the diet of both species, particularly for juveniles which were significantly depleted in $^{15}$N relative to female spiders. These results seem to conflict somewhat with observations of fly consumption based on gut content analyses of field-collected spiders (Chapter 3). The gut content survey indicated that all stages of both species regularly consumed flies in the litter layer throughout the course of the summer. There are several possible explanations for this discrepancy. One is that the observed frequency of fly consumption by spiders ($\approx$8% of total individuals) actually represents a small component of total prey consumed by the spiders. As we have little knowledge of spider predation rates in the forest it is difficult to form any conclusions regarding the validity of this hypothesis. It is possible, however, that flies provide spiders with a necessary resource (e.g. nutrient, amino acid, protein) which is limited in other prey groups. Such a scenario could lead to regular consumption of small quantities of flies in combination with larger quantities of other prey types. This idea is supported by laboratory rearing studies which have shown that spiders generally have higher fitness on mixed diets (e.g. Toft and Wise 1999a). A second explanation is that spiders may vary rates of ingestion and assimilation of
different prey types based on nutritional requirements. Mayntz et al. (2005) demonstrated that spiders can alter rates of extraction of carbon or nitrogen from prey to offset nutrient deficiencies. If forest-floor spiders consume flies in order to supplement a limiting nutrient under conditions of high resource availability, partial consumption of prey might be common. Conversely, some forest-floor flies, such as Sciaridae, might contain a feeding deterrent or otherwise be poor quality prey for spiders (e.g. Toft and Wise 1999b). Partial consumption of such prey may result under conditions of resource limitation, or in inexperienced spiders. One of the drawbacks to gut content analysis is that is difficult to account for partial consumption of prey by generalist predators. Gut content analysis provides detailed information about the rate of consumption of a prey group, but other techniques, such as stable isotope analysis, may be necessary to assess the importance of the prey in the predator's diet.
Table 5.1 Mean proportional contributions (±S.E.) and range of feasible proportions of prey contributed to the diets of *Schizocosa* spp. and *Anahita punctulata*

<table>
<thead>
<tr>
<th>Prey group</th>
<th>Schizocosa spp.</th>
<th>Anahita punctulata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Range</td>
</tr>
<tr>
<td>Adult ♀’s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detritivores</td>
<td>30.7 ± 19.9</td>
<td>0 - 71</td>
</tr>
<tr>
<td>Tomoceridae</td>
<td>28.1 ± 14.6</td>
<td>0 - 62</td>
</tr>
<tr>
<td>IG prey</td>
<td>15.0 ± 11.3</td>
<td>0 - 44</td>
</tr>
<tr>
<td>Misc. flies</td>
<td>9.7 ± 7.3</td>
<td>0 - 38</td>
</tr>
<tr>
<td>Fruit flies</td>
<td>10.8 ± 6.6</td>
<td>0 - 34</td>
</tr>
<tr>
<td>Herbivores</td>
<td>5.7 ± 4.2</td>
<td>0 - 42</td>
</tr>
<tr>
<td>Juveniles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomoceridae</td>
<td>56.7 ± 12.3</td>
<td>2 - 79</td>
</tr>
<tr>
<td>Detritivores</td>
<td>20.1 ± 17.1</td>
<td>0 - 97</td>
</tr>
<tr>
<td>IG prey</td>
<td>6.5 ± 5.8</td>
<td>0 - 37</td>
</tr>
<tr>
<td>Fruit flies</td>
<td>9.9 ± 3.7</td>
<td>0 - 22</td>
</tr>
<tr>
<td>Misc. flies</td>
<td>3.3 ± 3.1</td>
<td>0 - 20</td>
</tr>
<tr>
<td>Scuttle flies</td>
<td>1.8 ± 1.9</td>
<td>0 - 12</td>
</tr>
<tr>
<td>Herbivores</td>
<td>1.7 ± 1.8</td>
<td>0 - 11</td>
</tr>
</tbody>
</table>
Figure 5.1 Carbon:nitrogen ratios of forest-floor arthropods (±S.E.).
Figure 5.2 Stable isotope ratios for prey organisms from the detrital subweb (±S.E.). Circles represent groups of taxa that are not statistically different from one another. Fruit flies = Drosophilidae; Fungus gnats = Mycetophilidae, Sciaridae; IG prey = small spiders (< 0.1 mg); Predatory flies = Dolichopodidae; Scavengers = Anisopodidae, Calliphoridae, Phoridae, Sarcophagidae; 2 ° decomposers = Acheta domesticus, Gryllus sp. (Gryllidae), Entomobryidae (Collembola); Wood roaches = Parcoblatta sp. (Blattellidae).
Figure 5.3 Ontogenetic and size-related shifts in spider $\delta^{15}$N values for a) *Schizocosa* spp. ($y = 2.21x + 10.54$, $r^2 = 0.05 \varphi$ and $y = -1.44x + 7.42$, $r^2 = 0.13$ juvenile) and b) *Anahita punctulata* ($y = 0.95x + 10.78$, $r^2 = 0.12 \varphi$ and $0.25x + 3.89$, $r^2 = 0.01$ juvenile).
Figure 5.4 IsoSource mixing diagrams. The polygon indicated by the solid lines indicate the mixing space delineated by the source values. Separate models are presented for a) juveniles and b) adult females, with source groups restricted by minimum prey body size. \( Ana = Anahita punctulata, Sch = Schizocosa \) spp.
Figure 5.5 Proportional contributions of prey groups to the diets of juvenile *Schizocosa* spp.
Figure 5.6 Proportional contributions of prey groups to the diets of juvenile *Anahita punctulata*. 
Figure 5.7 Proportional contributions of prey groups to the diets of adult female *Schizocosa* spp.
Figure 5.8 Proportional contributions of prey groups to the diets of adult female Anahita punctulata.
Figure 5.9 Resource utilization by *Schizocosa* spp. and *Anahita punctulata*. Width of arrows represent estimated link strength. Dashed arrows indicate lack of information on the contribution of the prey group to the spiders' diets.
### APPENDIX A Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) for litter- and understory-dwelling arthropods (mean ± S.E.)

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Stage</th>
<th>Species</th>
<th>$\delta^{13}C$ 2006 ± S.E.</th>
<th>$\delta^{13}C$ 2007 ± S.E.</th>
<th>$\delta^{15}N$ 2006 ± S.E.</th>
<th>$\delta^{15}N$ 2007 ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dictyoptera</td>
<td>Blattellidae</td>
<td>Nymph</td>
<td>Parcoblatta spp.</td>
<td>-24.72 ± 0.41</td>
<td>-24.43 ± 0.15</td>
<td>-1.07 ± 0.94</td>
<td>-1.10 ± 0.28</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>Chrysomelidae</td>
<td>Adult</td>
<td></td>
<td>-25.64 ± 0.02</td>
<td>0.50 ± 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coleoptera</td>
<td>Curculionidae</td>
<td>Adult</td>
<td></td>
<td>-26.34 ± 0.39</td>
<td>0.27 ± 0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colembola</td>
<td>Entomobryidae</td>
<td>Mix</td>
<td></td>
<td>-27.21 ± 0.00</td>
<td>-26.12 ± 0.12</td>
<td>-6.08 ± 0.16</td>
<td>-1.09 ± 0.11</td>
</tr>
<tr>
<td>Colembola</td>
<td>Sminthuridae</td>
<td>Mix</td>
<td></td>
<td>-26.13 ± 0.10</td>
<td>-25.38 ± 0.08</td>
<td>-3.45 ± 0.58</td>
<td>-3.90 ± 0.14</td>
</tr>
<tr>
<td>Julida</td>
<td>Julidae</td>
<td>Mix</td>
<td></td>
<td>-24.19 ± 0.16</td>
<td>-25.04 ± 0.00</td>
<td>4.41 ± 0.00</td>
<td>7.13 ± 0.00</td>
</tr>
<tr>
<td>Diptera</td>
<td>Anisopodidae</td>
<td>Adult</td>
<td>Calliphora sp.</td>
<td>-25.64 ± 0.00</td>
<td>-25.04 ± 0.00</td>
<td>4.11 ± 0.00</td>
<td>7.13 ± 0.00</td>
</tr>
<tr>
<td>Diptera</td>
<td>Calliphoridae</td>
<td>Adult</td>
<td>Lucilia sp.</td>
<td>-25.07 ± 0.36</td>
<td></td>
<td>6.97 ± 0.58</td>
<td></td>
</tr>
<tr>
<td>Diptera</td>
<td>Culicidae</td>
<td>Adult</td>
<td>Aedes albopictus</td>
<td>-26.17 ± 0.00</td>
<td></td>
<td>0.01 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>Diptera</td>
<td>Dolichopodida</td>
<td>Adult</td>
<td></td>
<td>-25.22 ± 0.37</td>
<td></td>
<td>5.13 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Diptera</td>
<td>Drosophilidae</td>
<td>Adult</td>
<td>Drosophila sp. 1</td>
<td>-23.69 ± 0.37</td>
<td>-22.09 ± 0.24</td>
<td>11.80 ± 0.79</td>
<td>6.28 ± 0.92</td>
</tr>
<tr>
<td>Diptera</td>
<td>Drosophilidae</td>
<td>Adult</td>
<td>Drosophila sp. 2</td>
<td>-22.75 ± 0.10</td>
<td></td>
<td>5.50 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>Diptera</td>
<td>Muscidae</td>
<td>Adult</td>
<td></td>
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APPENDIX A (continued)
REFERENCES


VITA

Erin Elizabeth Hladilek

Date of birth: June 8, 1975
Place of birth: Menominee, Wisconsin

Education
• Ph.D. (expected May 2009), Entomology, University of Kentucky
• M.S. (2003), Entomology, University of Minnesota
• B.S. (1998), Zoology and Biological Aspects of Conservation, University of Wisconsin-Madison

Professional experience
• Graduate research assistant/Ph.D. candidate (2004 – 2008), Department of Entomology, University of Kentucky
• Senior Laboratory technician (2003 – 2004), Department of Entomology, University of Minnesota
• Teaching assistant (2001-2002), Department of Entomology, University of Minnesota
• Graduate research assistant (1999 – 2003), Department of Entomology, University of Minnesota
• Undergraduate research assistant (1998 – 1999), Department of Entomology, University of Wisconsin-Madison

Awards and honors
• Kentucky Opportunity Fellowship (2005), Department of Entomology, University of Kentucky
• Allan Peterson Award for Academic Achievement (2003), Department of Entomology, University of Minnesota

Publications