MECHANISMS AFFECTING PREDATION SUCCESS IN WOLF SPIDERS

by

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I've often heard it said that, "nothing worth doing is ever easy". This project was definitely not the exception. I fully believe that completing a graduate degree is a community effort, because the trial of graduate school tests both yourself, as well as those surrounding you. Over the past five years, I have poured my blood, sweat, and tears into this dissertation, and only with the support of an amazing group of mentors, colleagues, friends, and family has it finally reached its culmination. Without further due, here are the people that without which, my dream of teaching college biology would never have happened.

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Abstract

MECHANISMS AFFECTING PREDATION SUCCESS IN WOLF SPIDERS

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Predator-prey interactions play a role in community structure through a variety of different mechanisms including direct consumption, density-mediated indirect effects, and trait-mediated indirect effects. Understanding factors that can influence the efficacy of direct consumption on prey populations can help us to identify the strength of the impact predators can have on lower trophic levels. My dissertation research focuses on how mechanisms like sexual dimorphism, impaired body condition, and starvation stress alter how effective an intermediate predator (i.e. a predator that occupies an intermediate position in the food web) can be in direct consumption of prey. Using wolf spiders as my model, and a combination of laboratory experimental foraging trials and field experiment approaches, I assessed the effectiveness of spiders as predators. My main questions were how sexual dimorphism and limb autotomy affect predatory behaviors in wolf spiders. I also examined the effect of starvation on fitness and immunological trade-offs using biochemical immune assays This research reveals that female wolf spiders are more effective at direct consumption of prey than males due to a combination of body size, trophic morphology, and behavioral dimorphisms. Limb autotomy had no significant effect on foraging success in wolf spiders, although there was a trend of leg autotomy having a negative impact on prey capture ability. Lastly, starvation of spiders leads to an energetic trade-off where individuals appear to sacrifice physiological condition for

investment into immune defense. Findings from this dissertation can be utilized to help

further our understanding of how predator-prey interactions shape community structure.

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Introduction

1.1 Introduction to Predator-Prey Interactions

A community is an assemblage of interacting populations that coexist in an

ecosystem (Whittaker 1975). One of the most fundamental questions that ecologists

seek to answer is how the different organizational levels, for example individuals and

populations, interact to influence both population and community dynamics (Ovadia et al.

2007). Within a community, interactions between populations are responsible for shaping

food webs, influencing diversity, and determining community structure. These

interactions can range from positive to negative, and include competition, predation,

mutualism, and commensalism. Predation in particular is often considered one of the

driving forces that determine community structure as predators have tremendous

influence on other trophic levels (Beckerman et al. 1997, Morin 2011).

Predators can affect community structure in a variety of ways, however most

commonly, ecological theory views predator-prey interaction in terms of direct

consumption (Preisser et al. 2005). In this situation, the number of prey items in a

population decreases as predators consume them, thus altering prey population

abundance. Direct consumption is commonly considered to exert a top-down influence

on the community, as this standpoint assumes the community is most strongly influenced

by the strength of the interactions of higher trophic species on lower ones. This is also

the focus of many "vertical structure" models as well, where predator-prey interactions

contribute more toward community structure than competition for resources (i.e.,

horizontal structuring; Leibold et al. 1997). The top-down influence on community

structure has been studied extensively for several decades (Paine 1966, Robinson and

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Tonn 1989, Bizina 2000, Harvey et al. 2012). A classic example of top-down controls and the importance of predator-prey interactions in organizing community structure is the intertidal study involving the sea star *Pisaster* by Paine (1966). Paine found that when the keystone predator *Pisaster* was removed from intertidal plots for one year, species richness decreased. Without an important predator present in the community, dominant competitors excluded other species, decreasing species richness and altering the community structure (Paine 1966).

While prey abundance can decrease as a result of direct consumption by predators, the effect of the predators suppressing the prey population can also indirectly affect the functioning of third party trophic levels. If predators suppress a prey population by direct consumption, the decrease in the number of prey then results in an indirect, positive effect on the trophic levels below the prey, or a density-mediated indirect interaction (DMII; Werner and Anholt 1996, Preisser et al. 2005). Theoretical examples of a DMII include both trophic cascades and keystone predator effects (Paine 1966, Leibold 1989).

Predators are however not limited in their ability to alter community structure only by direct consumption. Often, simply the presence of predators can drastically alter prey population abundance through shifts in behavior or life histories, independent of direct consumption (Schmitz et al. 1997, Lima 1998, Peacor and Werner 2001, Schmitz 2007, Davenport et al. 2014). These nonlethal predation effects can result in dramatic changes in community structure. If there are local peaks of predator activity, this may result in prey modifying their behavior and exhibiting patch avoidance (Van de Meutter et al. 2005). This decrease in the abundance of available prey could then increase the rates of competition within the predator community, leading to more of an emphasis on horizontal structuring (Heithaus 2001, Birkhofer et al. 2011). Such a scenario is an example of a

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trait-mediated indirect interaction (TMII) in which one species modifies the way two other species interact by causing shifts in prey behavior or morphology. There is experimental evidence which suggests that effects due to TMII's may equal or even exceed the effects of direct consumption on prey populations (Preisser et al. 2005).

1.2 Predation Sequence

When attempting to understand how predators affect community dynamics, it can often be useful to look at predators in the context of a predation sequence. A predation sequence is a sequence of behaviors which a predator undergoes to consume prey (Eaton 1970, Gilbert and Rayor 1985, Wisenden et al. 1995, Berlin 2003). Each species of predator typically has a specific predation sequence with unique. However, all predation sequences at their most basic consist of four simple behaviors: search, pursue, subdue, and consume (Figure 1.1). That is, for a predator to successfully acquire energy, it must locate a suitable prey item, follow and pacify that prey item, and then ingest its prey. Because the completion of a predation sequence concludes with the direct consumption of prey, identifying mechanisms that can affect the outcome of such sequences can give insights into quantifying how effective a predator can be in field settings.
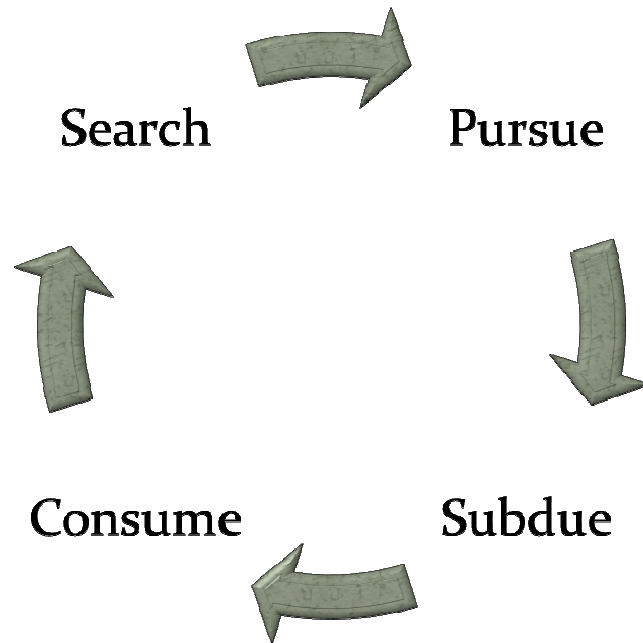
Figure 1.1 Example of a basic predation sequence. Predation sequences typically begin with a search phase and terminate in a consumption phase. Once the consumption phase has been completed, predators adopting a foraging strategy most likely return to a search phase, making the predation sequence circular.

1.3 Spiders Role in the Community

Spiders can contribute to the structure of the community through direct consumption. They are responsible for consuming a large proportion of the available biomass in many ecosystems. In a grassland ecosystem it was reported that wolf spiders (Lycosidae) alone consumed over 21% of the total net secondary production (Hook 1971). Spiders are integral in many terrestrial food webs by acting as multi-trophic level predators (Cronin et al. 2004). As many spiders exhibit polyphagy and consume a diverse arthropod diet, they are often considered to be important in reducing insect numbers (Hodge 1999, Rosenheim et al. 2004, Tahir and Butt 2009). Many spider species also prey upon one another as well as individuals of their own species, thus

increasing the complexity of the local food web (Balfour et al. 2003, Buddle et al. 2003, Schmidt-Entling and Siegenthaler 2009).

Not only do spiders act as predators on a variety of arthropods in the community, but they are also an integral portion of the diet of many higher trophic level predators (Wise 1995). For example, in some ecosystems spiders can make up 60-80% of local bird species diets (Hogstad 1984) and in many soil communities, spiders make up a substantial portion of the local biomass. One study of an olive grove ecosystem reported that over 20% of their field captures were arachnids, primarily members of Araneae (spiders) and Opiliones (harvestmen; Goncalves and Pereira 2012) Even more telling were the results of an abundance study in central California vineyards, where spiders constituted 98.1% of all predaceous arthropods collected (Costello and Daane 1999).

The presence of spiders in the community can alter the behavior of prey species. Prey may alter their foraging behavior (resulting in reduced feeding levels) in an attempt to decrease predation risk (Schmitz and Suttle 2001, Danner and Joern 2003). In fact, herbivorous arthropods have been shown to modify their diet choice based on the mobility of local spiders (Schmitz and Suttle 2001). Shifts in either diet choice or activity due to predator presence can have dramatic energetic consequences for spider prey species. For example, Western widow spiders (*Latrodectus hesperus*) commonly prey on harvester ants (*Pogonomyrmex rugosus*), and while they consume only 1.2 kcal of ant biomass per day, the ants in most cases cease foraging. This decrease in foraging activity results in a loss of 61 kcal per day for the ants (i.e. the ants lose more than 50 times as much energy by stopping foraging than by continuing to forage under predation pressure; MacKay 1982). The combination of decreasing prey population sizes through consumptive foraging or by indirectly causing behavioral shifts in herbivorous arthropods

allows spiders the ability to cause large scale changes in community structure (Cronin et al. 2004).

Simply labeling spiders as 'predators' may be an oversimplification of their role in the food web. Predators are often organized into guilds when classifying species in food webs. This approach can be a mistake as predator species in the same guild can often be segregated by habitat and can have significantly different capacities to capture and subdue prey (Schmitz and Suttle 2001). Because the outcome of single-prey, single-predator interactions are dictated primarily by the predator's hunting mode and the prey's microhabitat, it can be useful to categorize predators based on their foraging strategies (Schmitz 2005). It has been observed that cursorial hunting spiders (such as wolf spiders) often have significant direct consumption effects on their prey populations, possibly as a result of their active foraging strategy (Schmitz and Suttle 2001, Rosenheim et al. 2004). Active hunters like most wolf spiders are also more likely to reduce prey population densities than sit-and-wait predators like web building spiders (Schmitz 2009).

Cursorial spiders are known for being the dominant arthropod predator in a number of habitats (Muma 1973, Young and Lockley 1994, Samu and Szinetar 2002, Mallis and Hurd 2005, Marusik and Koponen 2005, Tahir et al. 2011). One species of wolf spider (*Pardosa littoralis*) has been found in field densities to frequently reach as high as 600 individuals per square meter (Finke and Denno 2002). Such high population values also allow them to effectively control local prey populations, as illustrated again by *P. littoralis* individuals that have been observed to consume up to 70 prey items in 24 hours (Döbel and Denno 1994). Due to their high biomass and key role in the trophic hierarchy in different ecosystems, understanding the variety of mechanisms that can affect predation success of wolf spiders could have important implications for how communities can be structured by intermediate trophic level predators. It is therefore

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necessary to understand how effective wolf spiders are as predators to ultimately identify how they impact community organization.

<p style="text-align:center">1.4 Dissertation Goals</p>

The overarching goal of this dissertation is to identify mechanisms that potentially alter how effective cursorial wolf spiders are at capturing prey. By understanding factors that can alter the outcome or expression of predation sequences, this dissertation can contribute to the understanding of active hunting, cursorial spider's role in organizing community structure and their influence on suppressing prey populations.

Chapter 2 – Sexual dimorphic characters and their impact on predator-prey interactions

Objective 1: To explore the effect of sexual dimorphism on prey capture ability in wolf spiders.

Objective 2: To compare a suite of morphological characters between male and female spiders hypothesized to affect prey capture success.

Objective 3: To ascertain if there are behavioral differences between sexes that account for differential success in prey capture.

Chapter 3 – Autotomy and its effects on wolf spider foraging success

Objective 1: To identify how body impairment affects predator success at capturing prey.

Objective 2: To assess if body impairment in both the predator and prey affects the outcome of a predation sequence.

Chapter 4 – The effect of food constraints on wolf spider (*Tigrosa helluo*) fitness

Objective 1: To identify how starvation affects physiological condition and immunity.

Objective 2: To discover if there is an energetic trade-off between physiological condition and immunity when spiders are energetically stressed.

Chapter 2

Sexually Dimorphic Characters and Their Impact on Predator-prey Interactions

2.1 Introduction

Sexual dimorphism is a phenomenon exhibited by many taxa where there is a difference in attributes between males and females of a species (Hedrick and Temeles 1989, Vollrath and Parker 1992). The evolution of sexual dimorphism is theorized to be a product of sexual selection (Head 1995), differences in reproductive roles (Fairbairn 1997), or intersexual niche divergence (Walker and Rypstra 2002, Beck et al. 2003). Sexual selection can result in characteristics becoming more distinct or elaborate either as a function of female preference (intersexual selection) or as a consequence of intrasexual selection in which males fight one another for mates (Greene and Funk 2009). Intersexual niche divergence can also drive the evolution of sexual dimorphism. If males and females occupy different niches, then they do not compete for the same resources, and both sexes can consequently maximize their energy uptake and fitness with few negative consequences to the other sex (Fairbairn 1997). Lastly, sexual dimorphism may be caused by the differentiation of reproductive roles. For example, among invertebrates, female size can influence clutch size and reproductive success. According to the reproductive roles hypothesis, female fitness should be maximized by increasing clutch size. Therefore, selection should act to increase body size since body size and clutch size are often correlated (Marshall and Gittleman 1994, Prenter et al. 1999). A sexual dimorphism could then result due to selective pressures being stronger for increased size in females but not in males.

Sexual dimorphisms can be expressed in a variety of different contexts, but are most commonly associated with overall body size, known as sexual size dimorphism

(SSD; del Castillo and Nunez-Farfan 2008). This body size dimorphism can be either male predominated (i.e. males are larger than females; most commonly illustrated in birds and mammals), or female predominated, which is most common among invertebrate taxa (Fairbairn 1997). Within the invertebrates, spiders are widely used as a classical model of female predominated SSD (Vollrath and Parker 1992, Head 1995, Prenter et al. 1998, Prenter et al. 1999, Walker and Rypstra 2003a), however the degree of SSD among spiders is highly variable (Head 1995). For example, web-building, sit-and-wait species have a higher degree of SSD, while species that are roaming predators tend to have less variation in morphological characters between the sexes (Vollrath and Parker 1992).

In addition to the morphological disparities between spider sexes, behavioral foraging adaptations can also vary (Holmes 1986, Clutton-Brock et al. 1987, Ginnett and Demment 1997, Beck et al. 2003). Males tend to be active foragers that traverse the environment in search of food and mates, while females are more frequently sedentary (Vollrath and Parker 1992). Wolf spiders of the family Lycosidae are typically active foragers that exhibit the stereotypical foraging strategy dichotomy found in most spiders (Prenter et al. 1998). For example, female *Tigrosa helluo* (former named *Hogna helluo*; Brady 2012) are known to build burrows and tend to be relatively sedentary, periodically changing their foraging locality (Stratton 1985). Adult male *T. helluo* on the other hand do not build burrows and actively search for prey as well as mates (Walker and Rypstra 2003b). As a function of dissimilar foraging patterns, the number of prey that each sex captures may differ as well.

These differences in male and female spider morphology and foraging behavior have the potential to impact predator-prey interactions, and thus fitness (Walker and Rypstra 2001). I used *T. helluo* (a lycosid with an extensive geographical range

commonly found in riparian habitats and agricultural fields; Walker et al. 1999) to investigate the effects of sexual dimorphism on predator-prey interactions. My goals were to (1) identify whether there were differences in foraging success between males and females, (2) determine which morphological characters contribute most to the differentiation in the sexes, and (3) ascertain whether there are behavioral adaptations between males and females in their foraging strategies. I hypothesized that due to differences in reproductive roles, female *T. helluo* would exhibit differences in trophic morphology and foraging strategies compared to males, resulting in female *T. helluo* capturing a significantly higher proportion of prey items than males.

2.2 Methods

Male (*n* = 85) and female (*n* = 100) *T. helluo* were captured by headlighting in Don Misenheimer Park (Arlington, TX) after dusk from 12 May to 3 October 2010. Specimen capture was conducted a total of seven times throughout the study period. To minimize the chance of a temporal bias, spiders from each collection date were evenly and randomly assigned a prey density of 1, 2, 4, or 6 crickets. For example, if twenty male spiders were collected in late May, each prey density was randomly assigned to five individuals from that collection date so that not all spiders captured at the same time period were assigned to one prey density. Spiders were maintained in the laboratory in clear plastic containers with a sand substrate as well as a one dram shell vial filled with water and stoppered with a cotton ball to provide moisture. Test organisms were maintained on a 12 hour reverse light:dark cycle to invert their nocturnal rhythm so that trials could be performed during the day. Spiders were fed one to two crickets (*Acheta domesticus*) weekly dependent on the size of the crickets. At each feeding, female *T. helluo* were checked for egg sacs. If an egg sac was present, it was removed from the spider before feeding. Spiders were given two weeks to acclimate to feeding and light

cycles prior to the start of trials. Each spider was starved for one week prior to their

assigned foraging trial, but after the acclimation period to standardize their hunger levels

and promote predatory behavior.

2.2.1 Foraging Trial Design

The day of each spider's assigned foraging trial, females were checked for egg

sacs. If females had an egg sac present, the sac was removed and the female was

randomly reassigned to a prey density. Females whose eggs sacs were removed were

not used in foraging trials for a minimum of one week to allow them to acclimate to the

loss of the egg sac. Foraging trials were conducted using round metal tins (25 cm wide x

9 cm deep) to avoid edge effects. Each chamber was filled with approximately two

centimeters of fine grade white sand to allow clear viewing of both predators and prey

during data collection. Sand used during trials was strained through a mesh screen to

eliminate drag line silk and consequently minimize pheromone deposits from interfering in

subsequent trials. Additionally, sand was only reused in trials of the same sex (e.g.

female sand was not used in male trials). A total of 185 trials were conducted from 3

June to 12 November 2010 (prey density 1 [males, $n = 21$; females, $n = 25$], prey density

2 [males, $n = 22$; females, $n = 26$), prey density 4 [males, $n = 20$; females, $n = 25$], prey

density 6 [males, $n = 22$; females, $n = 24$]). Each individual was run in one foraging trial

of their assigned prey density, and then was not used for subsequent foraging trials. All

crickets used as prey items were obtained from a same-sized cohort, and cricket prey

size was kept consistent across all trials and prey densities. To begin, the appropriate

number of crickets was introduced to the experimental chambers and allowed to

acclimate for approximately five minutes. The assigned *T. helluo* was then placed in the

center of the chamber in an inverted plastic vial for approximately ten minutes to allow for

acclimation. Once acclimated, the vials were removed and the spiders were allowed to

forage. Each chamber was then covered with a clear sheet of plexiglass to prevent the escape of spiders or prey items. Trials were conducted two at a time and recorded with a digital camera (Sony Handycam DCR-SR68) suspended above the experimental chambers for two hours after the initial movement of the spider. The proportion of prey items captured, as well as the time of prey capture for each prey item were recorded.

 2.2.2 Morphological Data Collection

Prior to each foraging trial, each spider had it's body mass recorded to the nearest mg using an analytical balance. After foraging trials, spiders were euthanized in 70% ethanol in preparation for morphological measurements. All anatomical variables were measured using digital microscopy and an image analysis program (Image J; National Institutes of Health). A variety of morphological variables were chosen based on characters that may affect foraging and/or prey capture, including the total length of leg I (front leg) and leg IV (back leg), pedipalp length, paturon (basal segment of the chelicerae; Kaston 1981) length and width, fang length, and carapace width (Rovner 1980, Walker and Rypstra 2001; Figure 2.1). For the legs and pedipalps, each segment of the appendage was measured separately and then these lengths were added to acquire the total length. The paturon width was measured from side to side halfway between the base of the chelicerae and where the fang attaches to the outer edge of the paturon. The length of the paturon was designated as the distance between the base of the chelicerae and the outer edge of the paturon, measured through the center. For each appendage measurement, either the left or right side was chosen randomly. Fang length was determined by measuring the linear distance between the center of the base of the fang where it attaches to the paturon and the tip of the fang. Lastly, carapace width was defined as the linear distance from one edge of the carapace to the opposite edge,

measured between legs II and leg III.
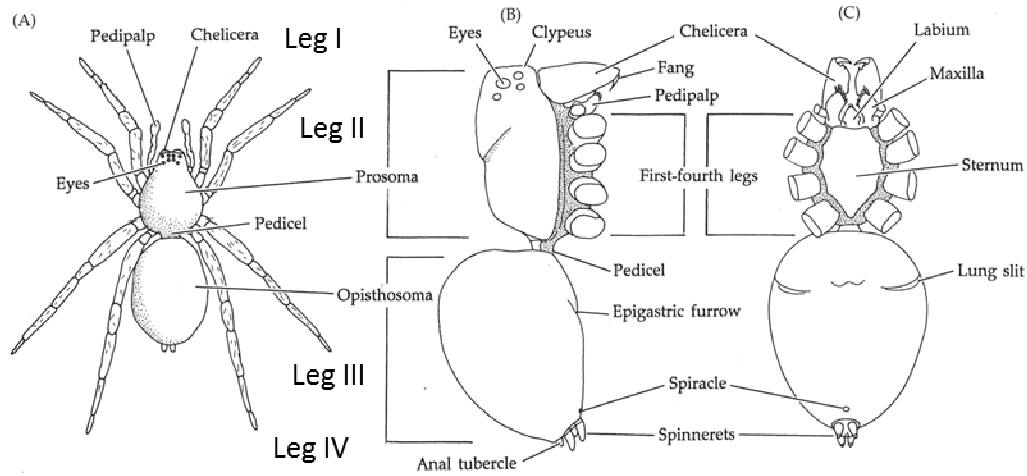
Figure 2.1 Generalized external spider anatomy. In this diagram, the structure labeled as the chelicerae can also be referred to as the paturon. The orientation is as follows: (A) dorsal view, (B) lateral view, and (C) ventral view (modified from Brusca and Brusca 2003).

2.2.3 Behavioral Data Collection

To identify if behavioral differences in foraging existed between the sexes, the total amount of time spent performing defined behaviors and the probability of transitioning between those behaviors was recorded using videos from the foraging trials. Only trials using six prey items were utilized for the behavioral analysis as they had the greatest differences between males and females in both the total proportion of prey items captured and the rate of prey capture. Timing for each video started when the spider first moved after recording began. Each video was then watched for 900 seconds (15 minutes) after initial movement, as the majority of prey captures occurred during this time period. Because only behaviors in which the spider was actively foraging were of interest, all trials in which the spider completed capture of all prey items in the chamber before 900 seconds elapsed were removed from analysis. One additional female trial

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was excluded as the female died shortly after the original video was collected. A total of 36 videos were viewed for behavioral data collection (males, $n = 16$; females, $n = 20$).

Behaviors were divided into six categories based upon preliminary observations: stationary, moving, reorienting, manipulation/repositioning, charge, and attack. Stationary behavior was observed when the spider was not moving in the experimental chamber. Spiders were considered to be moving if they were in active motion. This consisted of walking, running, and attempting to crawl up the sides of the experimental chamber. Reorienting was defined as occurring when the spider rotated in an arc while maintaining its position in the chamber. When the spider was handling prey or shifting position in place, the behavior was considered to be manipulating/repositioning. Manipulating/repositioning could occur at any time before or after prey capture. Spiders were defined as performing a charge when they rushed directionally toward a prey item, but did not make physical contact with the cricket. Lastly, an attack was characterized as occurring when the spider made physical contact with a prey item and attempted to grasp it with either its chelicerae or legs. The total amount of time spent performing each activity was recorded with the use of video editing software (Sony Vegas).

The probability of each individual to transition from one behavior to another was also calculated for integration into an ethogram. For each spider over the course of 900 seconds, the number of times they transitioned from one behavior to an alternative behavior was recorded. Each transition from one behavior to another was divided by the total number of transitions from the original behavior to determine the proportion of time the individual made those particular switches in behavior. These data were used to create a transition matrix for each spider, which were then separated by sex. Considering all possible transitions among the six defined behaviors, spiders could have exhibited a maximum of 30 possible unique behavioral transitions. Transitions that were performed

in less than 25% of the trials were considered rare and were not included in the construction of the ethogram. An additional two pathways were removed due to the presence of an extreme number of outliers (e.g. almost all non-zero proportions present for that transition were more than two standard deviations from the mean). The resulting ethogram had a total of 17 commonly observed behavioral transitions.

2.2.4 Statistical Analysis

The total proportion of prey items captured was compared between males and females using a Scheirer-Ray-Hare test (non-parametric two-way ANOVA; Sokal and Rohlf 1995, Dytham 2011) with sex and prey density as the main factors. The rate of prey survival (used as a proxy for the rate of prey capture by each *T. helluo*) was compared between males and females for each prey density using Kaplan-Meier survival analysis. The morphological characters examined included body mass, fang length, paturon width and length, total front and back leg length, and total pedipalp length. To assess what morphological characters were responsible for the greatest degree of sexual dimorphism between males and females, a multivariate analysis of variance (MANOVA) was conducted including all of the aforementioned characters. The amount of time spent performing each activity was compared between the sexes using *t*-tests with a Bonferroni correction to control for Type I errors. Time spent moving, reorienting, and performing charge behaviors were natural-log transformed to satisfy the assumption of normality. Lastly, transition probabilities were compared between sexes using either *t*-tests or, when in violation of normality, Mann-Whitney *U*'s. Transition probability comparisons were also subjected to a Bonferroni correction for multiple comparisons. All analyses were conducted using the Statistical Package for the Social Sciences Version 19 (IBM, Inc., 2010).

2.3 Results

Female spiders captured a higher proportion of prey at all prey densities than males. The difference in the proportion of prey captured between males and females increased with increasing prey density (Figure 2.2). The Scheirer-Ray-Hare test showed no significant interaction between sex and initial prey density (Table 2.1), and so the proportion of prey captured was compared between sexes for each prey density using Mann-Whitney U tests. For pairwise tests of the proportion of prey captured comparing male and female *T. helluo*, a Bonferroni correction was applied to the α-level to minimize inflating the risk of a Type I error. At low prey densities no significant differences in predation ability were detected between males and females (prey density 1 [males = 0.905 ± 0.066; females = 1 ± 0; U = 237.5; d.f. = 1, 44; P = 0.119] and prey density 2 [males = 0.841 ± 0.061; females = 0.942 ± 0.042; U = 232.0; d.f. = 1, 46; P = 0.084]; Figure 2.2). However, at higher cricket densities females captured a significantly higher proportion of available prey than males (prey density 4 [males = 0.650 ± 0.058; females = 0.910 ± 0.050; U = 96; d.f. 1, 43; P < 0.001] and prey density 6 [males 0.545 ± 0.061; females = 0.806 ± 0.063; U = 112.5; d.f. = 1, 44; P = 0.001]; Figure 2.2). At all prey densities, crickets survived at a higher rate when male *T. helluo* were the predators, than when female *T. helluo* were foraging (Figure 2.3). Kaplan-Meier survival analyses showed a significant difference in the rate of prey survival between male and female T. helluo for all prey densities (prey density 1 [Mantel-Cox test: Chi-square = 3.834, d.f. = 1, P = 0.050], prey density 2 [Mantel-Cox test: Chi-square = 6.724, d.f. = 1, P = 0.010], prey density 4 [Mantel-Cox test: Chi-square = 15.661, d.f. = 1, P < 0.001], prey density 6 [Mantel-Cox test: Chi-square = 27.055, d.f. = 1, P < 0.001]).
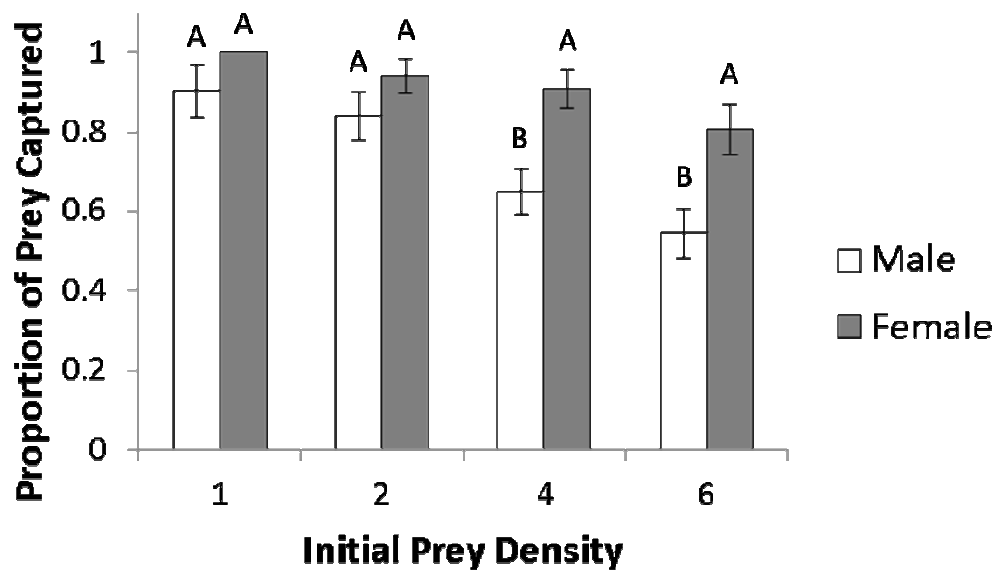
Figure 2.2 Average proportion of prey captured by male and female *T. helluo* at each prey density. Error bars represent the standard error of the mean and letters above each bar denote significant differences both between the sexes within each prey density and among each prey density.

Table 2.1 Results of the Scheirer-Ray-Hare test comparing the proportion of prey captured for each sex and initial prey density, as well as the interaction effect. *H*-values were obtained by calculating the total mean squares for a two-way ANOVA on the ranked data and dividing the corrected sum of squares (i.e. the sum of squares centered around the grand mean) by the respective degrees of freedom. *P*-values were calculated by testing the significance of each *H*-value as a χ^2 variable, also using the corresponding degrees of freedom (Sokal and Rohlf 1995, Dytham 2011).

	SS	d.f.	H	P
Sex	47703.282	1	24.35	<0.001
Prey Density	78833.474	3	40.24	<0.001
Sex*Prey Density	14344.248	3	7.32	0.062
Error	223154.218	177		
Total	171857.500	185		
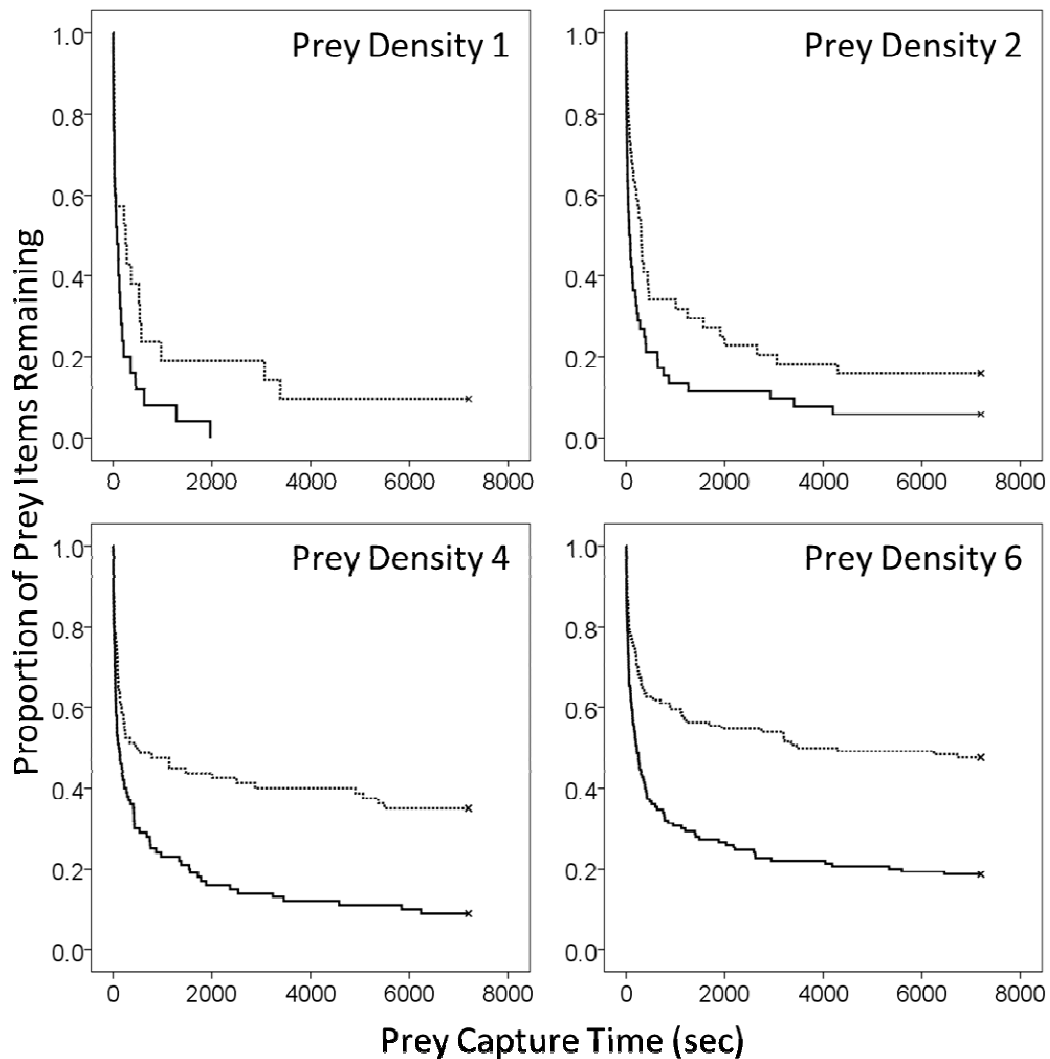
Figure 2.3 Comparisons of the proportion of prey items remaining over time for each cricket density. In each graph, the survival rate of prey when a female *T. helluo* was foraging is represented by the solid black line, and prey survival rates when male *T. helluo* were present is denoted by the dotted line. Crosses at the end of the survival curves indicate the presence of censored data in which not all prey items were captured by the end of the two-hour trials.

Females were heavier (males = 195.910 ± 8.640 mg; females = 373.062 ±

11.418 mg) and had significantly larger carapaces ([carapace length; males = 5.993 ±

0.101 mm; females = 7.334 ± 0.088 mm], [carapace width; males = 4.549 ± 0.075 mm;

females = 5.565 ± 0.073 mm]), fangs (males = 0.818 ± 0.019 mm; females = 1.171 ±

0.016 mm), paturons ([paturon width; males = 1.088 ± 0.026 mm; females = 1.643 ±

0.024 mm], [paturon length; males = 2.211 ± 0.043 mm; females = 2.891 ± 0.041 mm]),

and pedipalps (males = 6.177 ± 0.115 mm; females = 7.308 ± 0.084 mm) than males.

There were no significant differences in either front or back leg length between the sexes

(Table 2.2; Figure 2.4).

Table 2.2 MANOVA and univariate ANOVA results for each morphological character comparing male and female *T. helluo*.

	F	d.f	*P*
MANOVA			
Pillai's trace	5944.165	9, 168	**<0.001**
Univariate tests			
Spider mass	142.888	1, 176	**<0.001**
Carapace length	94.833	1, 176	**<0.001**
Carapace width	89.444	1, 176	**<0.001**
Fang length	215.029	1, 176	**<0.001**
Paturon length	126.726	1, 176	**<0.001**
Paturon width	251.733	1, 176	**<0.001**
Pedipalp length	63.549	1, 176	**<0.001**
Front leg length	3.501	1, 176	0.063
Back leg length	2.687	1, 176	0.103

P-values in bold are significant for the MANOVA at α = 0.05 and at α = 0.006 for the univariate ANOVA's.
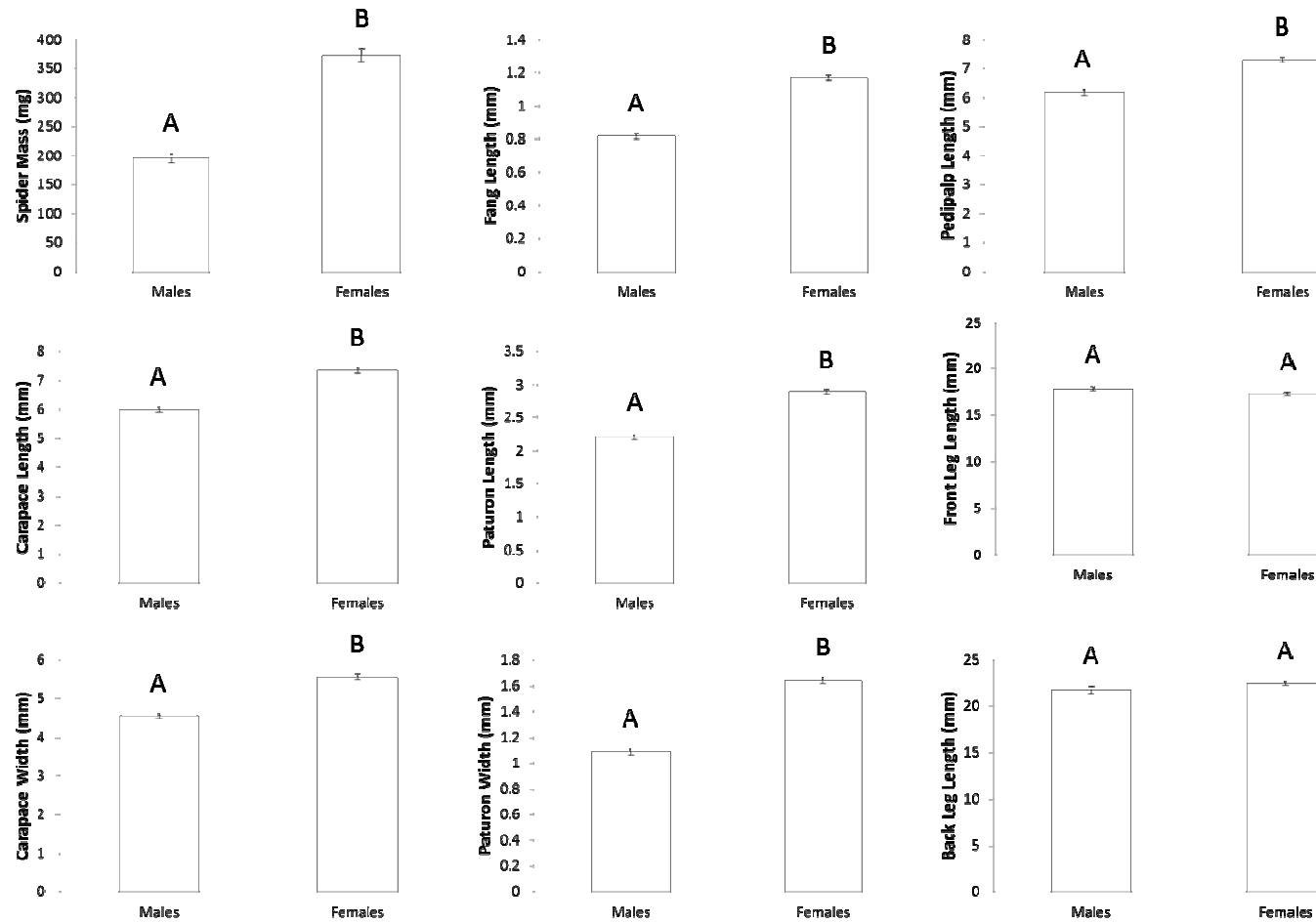
Figure 2.4 Comparisons of morphological characters (mean ± SE) for male and female *T. helluo*. Letters above the bars represent significant differences between the sexes for that morphological trait.

Females showed a trend of spending more time performing stationary (males = 682.867 ± 31.820 s; females = 698.947 ± 23.196 s), manipulating/repositioning (males = 62.750 ± 16.313 s; females = 85.611 ± 13.320 s), and charge (males = 1.429 ± 0.488 s; females = 2.800 ± 0.579 s) behaviors than males. Females also tended to spend considerably less time moving than males (males = 96.786 ± 15.393 s; females = 56.389 ± 12.673 s; Figure 2.5). After Bonferroni corrections however, male and female spiders did not spend a significantly different amount of time performing any of the observed behaviors (Table 2.3). The sexes did not show any significant differences in transitions between behaviors once the data were subjected to a multiple comparisons correction; however several strong trends in transitions between behaviors were present. Female spiders were much more likely on average to transition from stationary behavior to manipulating/repositioning and from attacking to manipulating/repositioning. However, males tended to transition from attacking to moving substantially more often than females did (Table 2.4; Figure 2.6)
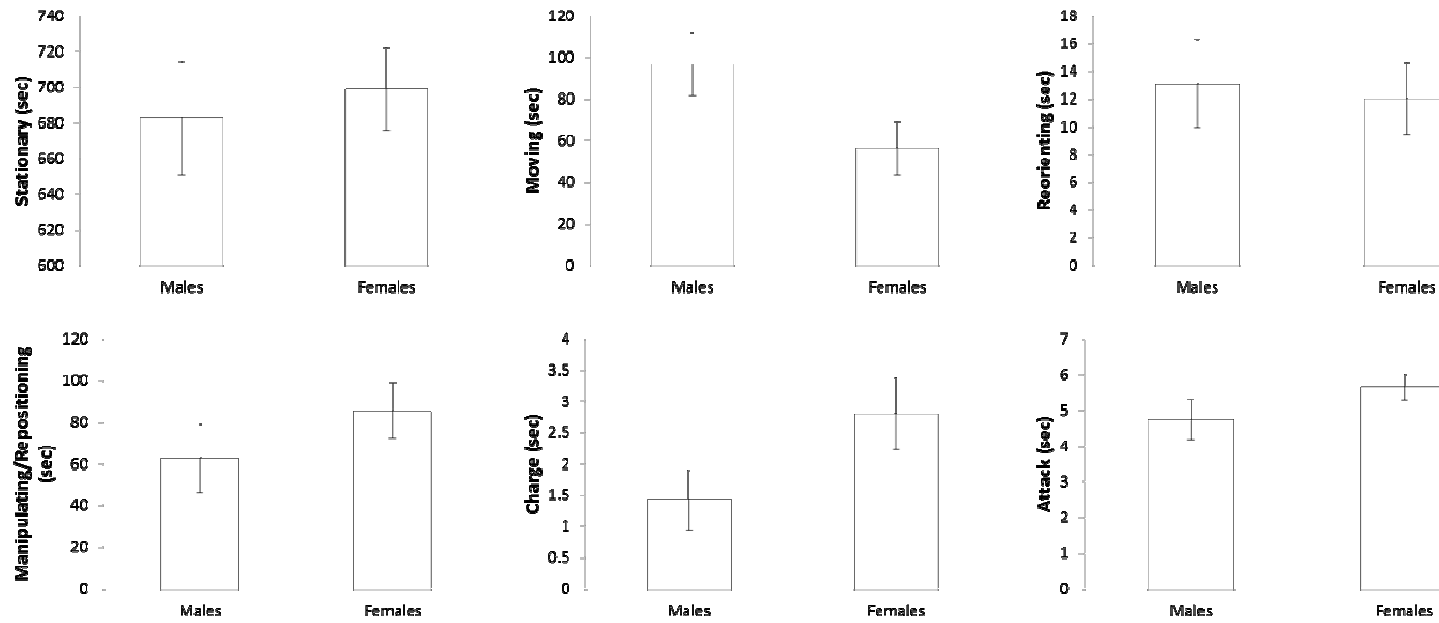
Figure 2.5 Comparisons of male and female *Tigrosa helluo* for the amount of time spent performing each behavior. Error bars represent the standard error of the mean.

Table 2.3 Descriptive statistics and *t*-test results for comparisons of the amount of time spent performing each behavior between male and female *Tigrosa helluo*. All comparisons were subjected to a Bonferroni correction resulting in an adjusted α-level of 0.0083.

| | Mean ± SE | | | | |
	Males	Females	*t*	d.f.	*P*
Stationary	682.867 ± 31.820	698.947 ± 23.196	0.418	1, 32	0.679
Moving	96.786 ± 15.393	56.389 ± 12.673	2.310	1, 30	0.028
Reorienting	13.143 ± 3.193	12.056 ± 2.600	0.179	1, 30	0.859
Manipulating/Repositioning	62.750 ± 16.313	85.611 ± 13.320	1.095	1, 32	0.282
Charge	1.429 ± 0.488	2.800 ± 0.579	1.655	1, 32	0.108
Attack	4.750 ± 0.574	5.650 ± 0.379	1.354	1, 34	0.185

Table 2.4 Descriptive statistics and *t*-test or Mann-Whitney *U* results comparing transition probabilities between male and female *Tigrosa helluo*. *P*-values were compared to a Bonferroni adjusted α-level of 0.0029 to identify statistical differences. Behavioral transitions with an asterisk were compared between sexes using a Mann-Whitney *U* test. All other comparisons utilized *t*-tests.

	Mean ± SE				
	Male	Female	*t* or *U*	d.f.	*P*
Stationary					
Stationary→Moving	0.489 ± 0.071	0.355 ± 0.055	1.520	1, 34	0.138
Stationary→Reorienting	0.111 ± 0.022	0.069 ± 0.016	1.564	1, 34	0.127
Stationary→Manipulating/Repositioning	0.284 ± 0.051	0.441 ± 0.047	2.273	1, 34	0.029
Stationary→Charge	0.033 ± 0.010	0.050 ± 0.010	1.266	1, 33	0.214
Stationary→Attack	0.059 ± 0.012	0.085 ± 0.008	1.871	1, 33	0.070
Moving					
Moving→Stationary*	0.919 ± 0.019	0.869 ± 0.032	118.0	1, 32	0.535
Moving→Manipulating/Repositioning*	0.033 ± 0.013	0.052 ± 0.016	129.0	1, 33	0.580
Moving→Attack*	0.025 ± 0.010	0.034 ± 0.011	142.0	1, 34	0.708
Reorienting					
Reorienting→Stationary	0.467 ± 0.098	0.436 ± 0.074	0.260	1, 34	0.797
Reorienting→Manipulating/Repositioning	0.099 ± 0.044	0.217 ± 0.054	1.678	1, 32	0.103
Manipulating/Repositioning					
Manipulating/Repositioning→Stationary	0.837 ± 0.034	0.841 ± 0.030	0.073	1, 31	0.942
Manipulating/Repositioning→Moving*	0.064 ± 0.020	0.044 ± 0.011	102.0	1, 30	0.536
Manipulating/Repositioning→Reorienting*	0.041 ± 0.020	0.068 ± 0.014	89.50	1, 33	
Charge					
Charge→Stationary*	0.474 ± 0.114	0.576 ± 0.089	142.0	1, 35	0.551
Attack					
Attack→Stationary	0.366 ± 0.061	0.306 ± 0.050	0.776	1, 32	0.444
Attack→Moving*	0.125 ± 0.038	0.026 ± 0.019	71.50	1, 31	0.012
Attack→Manipulating/Repositioning	0.399 ± 0.067	0.610 ± 0.064	2.253	1, 34	0.031
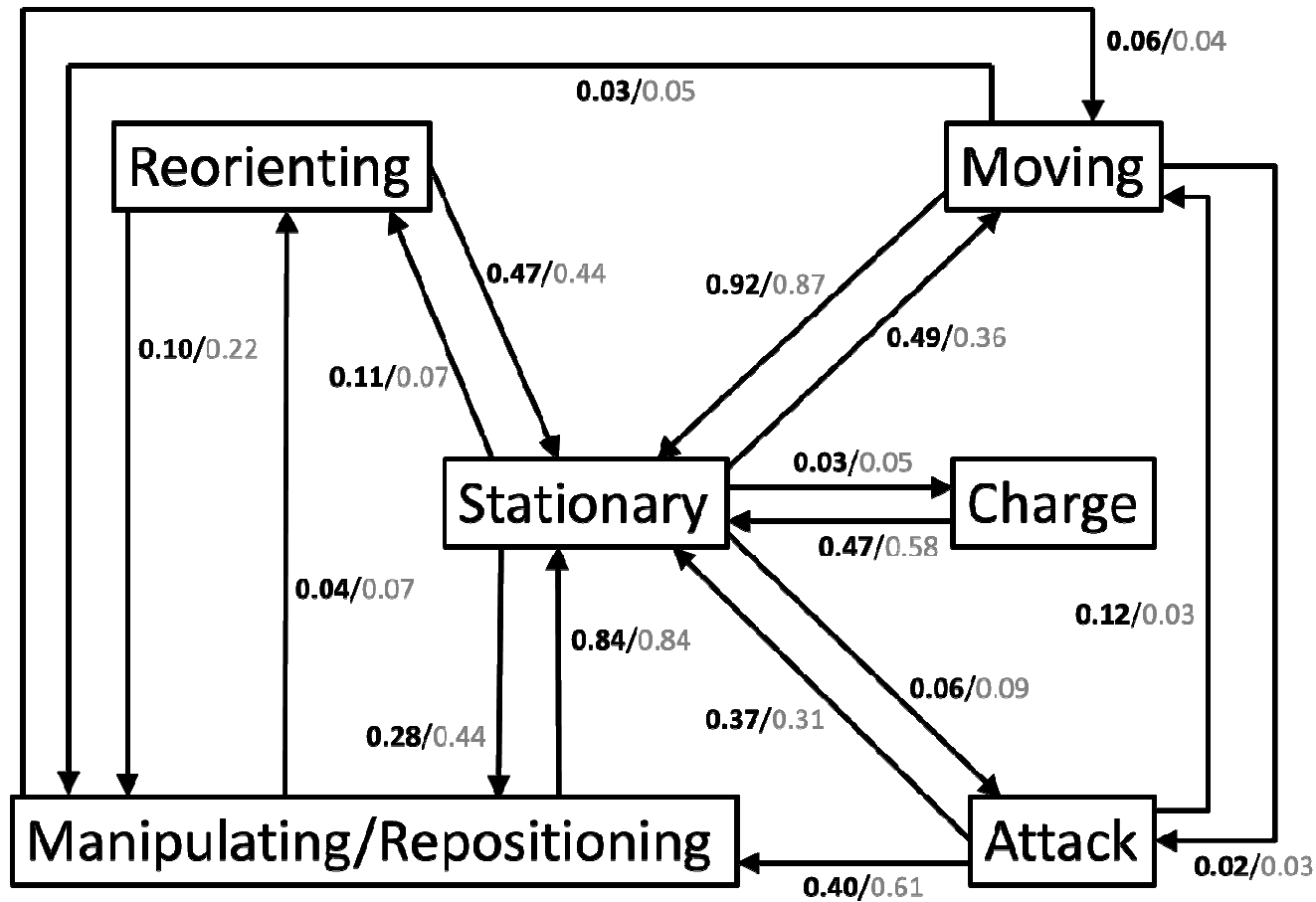
Figure 2.6 Ethogram of major behaviors exhibited by *Tigrosa helluo* during foraging trials. Values for each arrow represent the probability of transitioning from one behavior to another in the direction of the arrow. Numbers before and after the slashes denote the mean male and female transition probabilities, respectively.

2.4 Discussion

Sexual dimorphism may have evolved as a result of a variety of different mechanisms. The most common hypotheses include sexual selection for male size, intersexual niche divergence to minimize competition among the sexes for shared resources, or differences in reproductive roles (i.e. fecundity selection) leading to dimorphisms that maximize energy acquisition for reproducing females and mate encounters for males (Head 1995). In this study, I hypothesized that female *T. helluo* would capture more prey, and have enlarged trophic characteristics compared to males as a result of differences in reproductive roles or intersexual niche divergence.

Female *T. helluo* captured a higher proportion of prey items at higher prey densities than males, but this relationship was not significant at low prey densities. Survival analyses indicated that as prey density increased, the differences in the rate of prey survival between male and female *T. helluo* treatments also increased, possibly explaining why females captured a higher proportion of prey than males. Females capturing or consuming more food than conspecific males has been demonstrated several times in spiders (Walker and Rypstra 2001, 2002), as well as in a variety of other taxa. In many groups other than spiders, females are often shown to consume more food (Boinski 1988), invest more effort in foraging than males (King and Duvall 1990, Ginnett and Demment 1997, González-Solís et al. 2000), or be more selective in their dietary choices (Boinski 1988, Stokke 1999).

Overall, female *T. helluo* were larger bodied than their male counterparts. Among invertebrates, female size is often directly correlated with fecundity (McLain et al. 1990, Roff 1992, Berger et al. 2008). Therefore, if fecundity selection is active in a given population, female size should increase independent of changes in male size (Prenter et al. 1999). Body size itself can influence the amount and rate of food acquired (Andersen

and Saether 1992), and has been shown to affect foraging success in spiders as well (Head 1995). Based upon this relationship, the larger sex should be predicted to capture and consume the most prey items, as observed in my study. Females also had significantly larger chelicerae, and longer pedipalps and fangs than males. The chelicerae and fangs are used almost exclusively for prey capture in wolf spiders. While the pedipalps are used in courtship by males, they also play a critical role in prey capture in both sexes. Spiders often use a combination of their chelicerae and pedipalps to manipulate prey items and to create a food ball (Foelix 2010). Pedipalp coxae (the proximal segment of the pedipalp) are also highly specialized for crushing prey items while feeding (Foelix 2010, Herberstein 2011). Because of the energetic costs associated with reproduction, energy acquisition is most likely more important for females (Walker and Rypstra 2002). Therefore, morphological traits that confer an advantage for foraging may be selected for in females as a result of differences in reproductive roles between the sexes or intersexual niche divergence (Walker and Rypstra 2002).

Though measures of body size and trophic morphology showed differentiation between males and females, the length of both the front and back legs did not. Leg morphology was chosen to examine due to the role of legs I (front pair) and IV (back pair) in the prey capture process. When capturing prey items, wandering spiders will typically use their legs IV to thrust themselves closer to prey, while their legs I are used to grasp and manipulate the prey item (Rovner 1980). However, while the trophic characteristics are used most commonly in prey capture (and so should be most affected by selective pressures associated with foraging), leg morphology may also be affected by selective pressures not associated with prey capture (e.g. courtship displays; Hebets and Uetz 2000). It is therefore not surprising that we found significant sexual dimorphism in both body size and trophic characteristics, but did not find such differences in leg length.

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While there were no statistically significant differences between the sexes in either time budgets or transition probabilities, there were several noticeable trends. Female spiders tended to spend less time moving than males, while males were far more likely to shift from attacking to moving. These results concur with Walker and Irwin (2006), indicating that female *T. helluo* appear to be more sedentary, while males are typically more active and spend more time moving than females. Females are perhaps more sedentary because remaining stationary may increase their encounter rate with prey items. Beck et al. (2003) postulated that because females need substantial reserves of energy to allocate toward reproduction, they may alter foraging patterns to increase energy acquisition. Males however may be reducing the amount of time spent on foraging to allow more time for other activities, such as searching for mates (Boinski 1988, Ginnett and Demment 1997).

Both the amount of time spent attacking and charging, and the increased propensity to shift from stationary to attacking compared to males, points toward females being the more aggressive of the two sexes. This perceived aggressiveness could potentially contribute toward females capturing a higher proportion of prey items as aggression can contribute to foraging success (Butler and Stein 1985). I also observed that switching to manipulating/repositioning from other behaviors was more probable for females, and females spent more time performing manipulating/repositioning behavior. Spending more time manipulating captured prey items is another potential mechanism contributing to differential prey capture by the sexes. If female *T. helluo* spend more time forming prey into a more manageable food ball, then they could be more successful at catching and maintaining their hold on subsequent prey items. Ultimately, such differences in foraging strategies have been proposed as important mechanisms in the evolution of sexual dimorphisms (González-Solís et al. 2000).

This differential success in prey capture could likely result from different selective pressures on the sexes (Walker and Rypstra 2002). Females often must only be concerned with searching for food resources. Males, however, must search for and locate two stations in time and space, food and mates (King and Duvall 1990). Because males must split their time between two aims, they must budget their time accordingly. In the context of foraging, males can be thought of as time minimizers, while females can be categorized as energy maximizers. Because females must acquire and expend much more energy in reproduction than males, they must optimize their foraging success. Males must balance their time between energy acquisition and reproductive competition (Wrangham and Smuts 1980). Divergence of these behavioral strategies could then allow selection to act toward further differentiation in morphological traits, resulting in further divergence of behavioral strategies and foraging outcomes.

Chapter 3

Autotomy and its Effects on Wolf Spider Foraging Success

3.1 Introduction

Physical injury can be examined in the context of missing a body part, and has

the potential to affect the ability of a predator to capture prey items in a variety of ways.

For example, losing a body part has been shown to decrease the running speed of a

variety of different species (Bateman and Fleming 2009). Since many species actively

forage for prey, this decrease in locomotive efficiency can have dramatic energetic costs

if prey can more easily escape the affected individual. In addition to decreased

movement capability, there can be behavioral consequences to losing body parts. For

example, damselfly larvae that have lost caudal lamellae have been shown to be

significantly less active or aggressive than those with intact appendages (Stoks 1999).

Such declines in activity can then also lead to decreased foraging rates (Maginnis 2006).

Many species can voluntarily lose body parts through the use of autotomy. This

technique has been shown to be an effective predator escape mechanism in many

different taxa (e.g. spiders [Amaya et al. 2001], sea stars [Gaymer and Himmelman

2008], salamanders [Marvin 2010], insects [Bateman and Fleming 2011], lizards [McElroy

2011], crabs [McLain and Pratt 2011]). While the frequency of autotomy as a defensive

strategy is well documented in the literature (e.g. over 200 groups of invertebrates are

known to utilize autotomy; Fleming et al. 2007) many of the consequences of this action

are not.

The autotomy of a limb has the potential short-term benefit of helping to escape

a predator, and is used frequently by some groups of spiders. For example, spiders of

the genus *Filistata* will autotomize one or more of their legs when attacked by a scorpion

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(Formanowicz 1990). This action serves to distract the predator and provide an opportunity for the spider to escape. Even if the spider is successful, autotomy may still have long-term consequences that decrease the overall fitness of the organism compared to intact individuals (Fleming et al. 2007).

Autotomy has also been hypothesized to affect fitness in spiders by having a negative effect on foraging ability and success. Losing a limb can limit locomotive proficiency, as spiders with fewer than eight legs have been shown to have significantly reduced running speeds (Amaya et al. 2001, Apontes and Brown 2005, Brown and Formanowicz 2012). Limb loss may also affect the ability of a spider to capture prey, although of the studies that have tested foraging success in autotomized spiders, results have been conflicting. Amaya et al. (2001) showed that in two species of wolf spiders (*Varacosa terricola* and *Schizocosa ocreata*) there was no difference in the number of attacks necessary to capture prey items between intact and autotomized individuals, while Brueseke et al. (2001) found that autotomized *Pardosa milvina* tended to consume smaller crickets than their intact counterparts. However, Wrinn and Uetz (2008) found that *S. ocreata* individuals with autotomized legs had reduced prey capture rates when tested on a heterogeneous, natural substrate.

What has not been tested is how the loss of specific legs affects the outcome of predation events. Spider legs are numbered in pairs, with the first pair (legs I) being the most anterior and the fourth pair (legs IV) being the most posterior (Figure 2.1). When spiders attack prey items, each set of walking legs has a different function; however, of particular importance are legs I and IV. Wolf spiders typically leap short distances to attack prey. First, they raise legs I in a high arc, and then legs II are similarly raised. The spider will then push forward and obliquely upward with legs IV, which provide most of the propulsion for leaping toward the prey item. Legs I and II make initial contact with the

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prey and the spider will then use its posterior set of walking legs (III and IV) to move closer to the prey for venom injection (Rovner 1980). Because these legs have different functions, losing, for example leg I versus leg IV should have different effects on their long-term foraging efficiency.

Spiders in many communities commonly participate in unidirectional intraguild predation, where both species will prey on lower trophic levels, but one species functions as a top predator, and the other is considered to be an intermediate predator (Wise 1995, Vance-Chalcraft et al. 2007). Such unidirectional intraguild predation has important implications for community structure and thus it is critical to understand factors that can affect the outcome of such predation events (Vance-Chalcraft et al. 2007). When both intraguild predator and intraguild prey are capable of autotomy in such communities, it provides an opportunity to study the effects of diminished body condition on both predator and prey simultaneously. Furthermore, it provides insight into the effects of individual body condition on complex trophic dynamics. In the present study, I used two riparian-zone wolf spiders from southeastern Arizona (*Rabidosa santrita* and *Pardosa valens*) which both autotomize legs and which are involved in an intraguild predatory relationship. Our goals were to (1) test whether the specific leg autotomized had different effects on foraging success and (2) identify how impaired body condition in both predator and prey affects the outcome of predation events.

<div align="center">3.2 Methods</div>

3.2.1 Study Area and Specimen Collection

Cave Creek is a small stream located in the Chiricahua Mountains of southeastern Arizona, USA. Two wolf spiders species coexist in the wet and dry cobble zone of Cave Creek, both at relatively high densities. The smaller species, *P. valens* (30 – 140 mg adult mass), is active on the surface both during the day and night. The larger

species, *R. santrita* (375 – 1000 mg adult mass), generally emerges after sunset and spends the day under rocks. Adult and larger juvenile *R. santrita* are capable of eating all sizes of *P. valens*, and will hereafter be referred to as the predator. Although we have not observed this, adult *P. valens* can likely eat only the smaller instars of *R. santrita*; in this study *P. valens* will hereafter be referred to as the prey.

All spiders of both species used in the experiments were captured from an area of Cave Creek adjacent to the Southwestern Research Station (SWRS; Cochise Co., AZ; altitude 1620 m) between 1 June and 8 June 2012. To identify the frequency of predators exhibiting autotomy in field settings, we spotlighted for predators on 1 June 2012 for four search hours. A search hour was defined as one researcher searching for one hour. All predators that were spotted were captured if possible ($n = 119$). The number of individuals that were missing one or more legs was counted to obtain the proportion of predators in the population that showed evidence of autotomy. Sampling was performed again on 12 June 2012 for four search hours to identify which legs were most commonly autotomized by the predators. All predators spotted that were missing one or more legs were captured ($n = 18$) and the position(s) and number of missing legs were recorded for each individual.

3.2.2 Experimental Trials

For experimental trials, adult female predators were captured at night using head lamps to locate spiders via their eye shine, while prey were captured during the day by flipping rocks in the creek bed. For both species, only adult females with all legs present were used in trials. All spiders were maintained in the entomology lab at the SWRS in plastic centrifuge tubes (50 ml for predators and 15 ml for prey). Individuals were provided with moisture by placing a damp cotton ball in the mouth of the tube, and the lab was exposed to ambient light and temperature regimes. All spiders (both predators and

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prey) were kept under laboratory conditions no more than two days until participating in a predation trial. Care was taken to minimize disturbance of the specimens as much as possible prior to predation trials to reduce the effect of stress.

On the day following collection, the pre-foraging trial running speed of each predator was determined. Running speed was obtained by testing spiders along a 1 m track, constructed from a piece of transparent plastic tubing cut in half lengthwise and then glued to a piece of wood. Intervals of 0.5 m were marked on the plastic, with a small holding chamber adjacent to the beginning of the track. The holding chambers consisted of an additional 10 cm of plastic tubing that could be blocked at either end with removable index cards. Spiders were placed in the holding chamber and allowed to acclimate for approximately five minutes. After acclimation, the gates were removed and the specimens were prompted to run by chasing them down the track using a wooden dowel with a sponge attached to it (Amaya et al. 2001). The dowel was constantly pushed behind the spider such that if the spider stopped running the sponge would immediately brush their back legs and prompt the individual to continue running; however, the sponge was not used to push the spider. Each trial was recorded using a Sony Handycam DCR-SR68, and the time that spiders ran each 0.5 m segment was documented using video editing software (Sony Vegas Movie Studio 2009). The speed (in m/s) was then calculated by taking the inverse of total time each spider took to run the full meter.

Following running speed trials, predators were randomly assigned to one of two groups. In the first group, all of the prey provided to the predators were intact, and in the second group, all of the prey had one randomly-selected leg IV autotomized. In each prey group, the predators were randomly assigned to one of three treatments: control, in which no legs were autotomized (intact prey, $n = 17$; autotomized prey, $n = 18$); leg I autotomized (intact prey, $n = 11$; autotomized prey, $n = 11$); and leg IV autotomized

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(intact prey, n = 11; autotomized prey, n = 12). Both predators and prey in each experimental group had their egg sac removed if present (approximately 15% of individuals of both species had egg sacs present; personal observation), and then had autotomy of the appropriate leg induced. To induce autotomy, the femur of the appropriate leg was grasped with forceps and constant pressure was applied until the spider autotomized the leg. Predators were then returned to their centrifuge tube, offered one intact prey item, and given 24 hours before the start of predation trials to standardize hunger levels and to recover from leg loss. Prey species were also returned to their centrifuge tube and allowed to recover for 24 hours before foraging trials, but were not fed.

Foraging trials were conducted from 4-10 June 2012. Round metal tins (25 cm diameter x 9 cm deep) were used for the predation trials and were sterilized with alcohol before each trial to eliminate potential chemical cues. Each chamber was filled with 1-2 centimeters of local rock scree. Large rocks were removed from the scree to make a relatively homogeneous chamber surface. New rock scree was used for each trial. Each spider (for both the predator and prey) was examined prior to the predation trial and was not used if they had either autotomized an extra leg or produced an egg sac during the 24 hour acclimation period. Unequal sample sizes resulted from the elimination of these individuals prior to the commencement of foraging trials. All trials began after dusk, in the open air outside of the SWRS entomology lab, and had no cover on the chambers to simulate natural conditions as closely as possible. Five prey were placed in a chamber and allowed to roam freely, while one predator was placed in an upside down vial at the center of the chamber. Both predator and prey were then allowed to acclimate for approximately 10 minutes. After acclimation, the vial was removed and the predator was allowed to forage at its discretion. Using a red light to minimize the effect of human

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presence, chambers were checked every 15 minutes for one hour, and the number of

surviving prey present was recorded at each time interval. Twelve predator trials were

run at the same time with the start times staggered at one minute intervals. At the end of

the trial, all predators and prey were recaptured in vials. The prey remained in captivity

until all trials were completed, and were then released into the field. The day after

predation trials, all predators had their post-foraging trial running speed recorded using

the previously described methods. All predator spiders were then euthanized by placing

them in a freezer for approximately 4 hours. Following euthanasia, specimens were

preserved in 95% ethanol.

3.2.3 Statistical Analysis

Spider running speeds were analyzed using a single-factor repeated measure

ANOVA with the predator leg treatment as the main effect and pre- vs. post-autotomy

speed as the repeated measure. The total proportion of prey captured by the predators

was compared among experimental treatments using a non-parametric two-way ANOVA

(Scheirer-Ray-Hare test; Dytham 2011), as the analysis was done on count data (Amaya

et al. 2001). Kaplan-Meier survival analysis was used to compare the rate of prey

survival among the predator treatment groups when all prey were intact. Survival

analyses were again used to compare prey survival rates when prey were intact versus

autotomized within similar predator leg treatments. All analyses were performed using

the SPSS version 19.0 computer package (SPSS 2010).

3.3 Results

Of the 119 predators captured on 1 June 2012 to identify the frequency of

autotomy in the field, 18 individuals (15.13%) were missing one or more legs. Following

sampling on 12 June 2012, it was determined that legs IV were the most commonly

autotomized, followed closely by legs I (Table 3.1). Of the 18 individuals who showed

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evidence of autotomy, seven (38.89%) had more than one leg missing. No spiders
captured in this survey had more than two legs missing.

An outlier labeling test was performed on both the running speed and predation
data, and all outliers were removed from analyses. To begin, the interquartile range
(75th-25th percentile) of each variable tested was multiplied by a constant, in this case
2.2 (Tukey 1977, Hoaglin and Iglewicz 1987). This calculated value was then added to
the 75th percentile to create an upper fence and subtracted from the 25th percentile to
obtain a lower fence boundary. Any value outside of these fences was considered to be
an outlier (Hodge and Austin 2004, Schwertman et al. 2004). This test was utilized as it
is much less sensitive to extreme outliers than simply identifying outliers as observations
more than two standard deviations from the mean of the distribution (Seo 2006). Four
cases from the running speed data and three cases from the predation trial data were
identified as outliers and removed from all analyses. Running speeds were natural-log
transformed to satisfy the assumptions of the repeated-measures ANOVA. There were
no significant differences in running speed among predator foraging groups ($F_{2,40}$ =
0.110; P = 0.896) or between the pre-and post-foraging running speeds ($F_{1,40}$ = 1.394; P
= 0.245). However, individuals that had either a leg I or IV autotomized trended toward
having slower running speeds than their respective controls (Figure 3.1). No significant
interaction between leg treatment and pre- vs. post-foraging was detected ($F_{2,40}$ = 0.459;
P = 0.635).

Table 3.1 The frequency of autotomy by walking leg in *Rabidosa santrita* (predator).
Values represent the total number of instances the corresponding leg was not present
within the sample of autotomized individuals (n = 18), and the resulting percentage. The
total number of legs missing is greater than the number of individuals captured due to
some individuals (n = 7) missing more than one leg.

	Leg Lost			
	I	II	III	IV
Count	7	4	6	8
Percentage	28	16	24	32
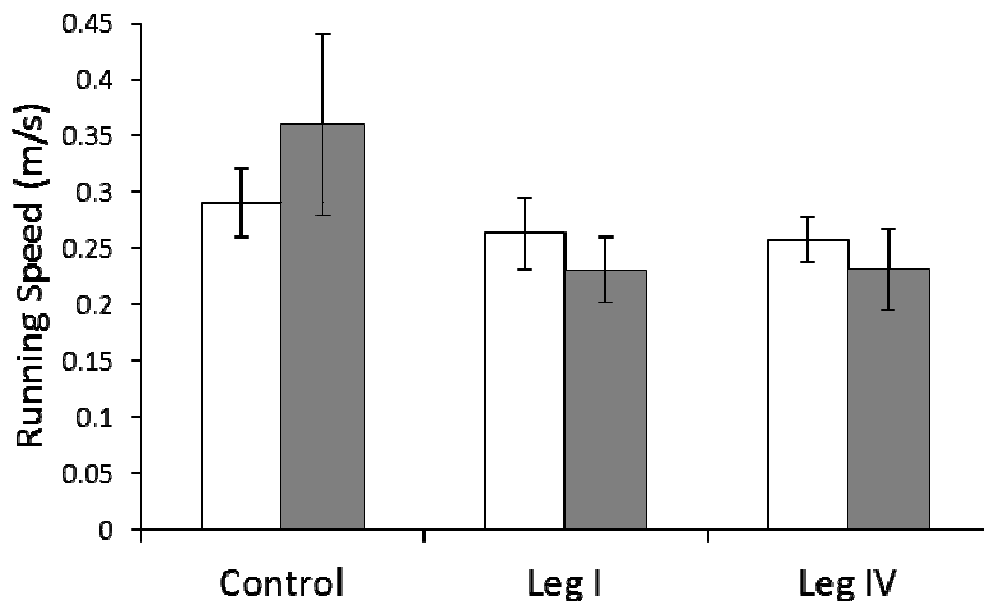
Figure 3.1 Comparisons of the pre- and post-foraging trial running speeds for each of the
predator leg removal treatments. White bars represent pre-trial running speed (when all
spiders had legs intact) and gray bars represent post-trial running speed (where control
spiders had all legs intact, but both leg I and IV bars had the appropriate leg
autotomized). The error bars signify the standard error of the mean.

There was no significant effect of either predator or prey leg treatments on the

total proportion of prey captured, nor was there a significant interaction between the two

factors (Table 3.2). When prey were intact however, there was a trend of control

predators catching the highest proportion of prey, individuals missing leg I catching the

next highest proportion, and individuals missing leg IV catching the lowest proportion of

the total available prey (Figure 3.2). The Kaplan-Meier survival analysis comparing

predator groups where all prey were intact showed a moderately significant difference

(Mantel-Cox test: χ^2 = 5.149, d.f. = 2, P = 0.076; Figure 3.3a). There were no significant

differences in prey survival rate comparing intact versus leg IV autotomized prey for

either the control (Mantel-Cox test: χ^2 = 0.089, d.f. = 1, P = 0.766; Figure 3.3b) or leg I

autotomy predator groups (Mantel-Cox test: χ^2 = 1.489, d.f. = 1, P = 0.222; Figure 3.3c).

When predators had a leg IV autotomized, there was a moderately significant difference

in prey survival rates (Mantel-Cox test: χ^2 = 3.519, d.f. = 1, P = 0.061). Intact prey tended

to survive at a higher rate than leg IV autotomized prey (Figure 3.3d).

Table 3.2 Results of the Scheirer-Ray-Hare test comparing the rank proportion of prey captured among predator and prey leg treatments. MS Corrected total is used in place of the mean square in a Scheirer-Ray-Hare test and is calculated by dividing the sum of squares of the corrected total row by the corrected total degrees of freedom (MS Corrected total = 514.399). The appropriate P-values were obtained for each main factor and the interaction effect by determining the cumulative probability that a value from the chi-square distribution with the corresponding degrees of freedom would be less than the value for each H statistic (Dytham 2011).

	SS	d.f.	H	P
Predator Leg Treatment	2311.110	2	4.49	0.11
Prey Leg Treatment	1471.217	1	2.86	0.09
Interaction	585.689	2	1.14	0.57
Error	36636.065	74		
Total	171857.500	80		
Corrected Total	40637.500	79		
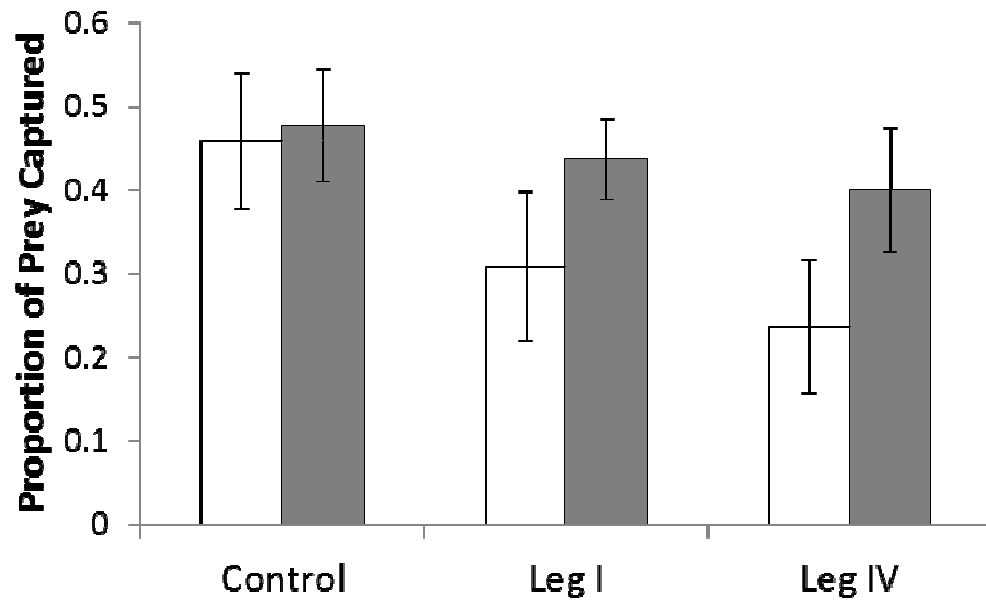
Figure 3.2 Bar graphs comparing the total proportion of prey captured among predator treatment groups. White bars represent trials in which prey were intact and grey bars represent trials where each prey item had a randomly chosen leg IV autotomized. Error bars signify the standard error of the mean.
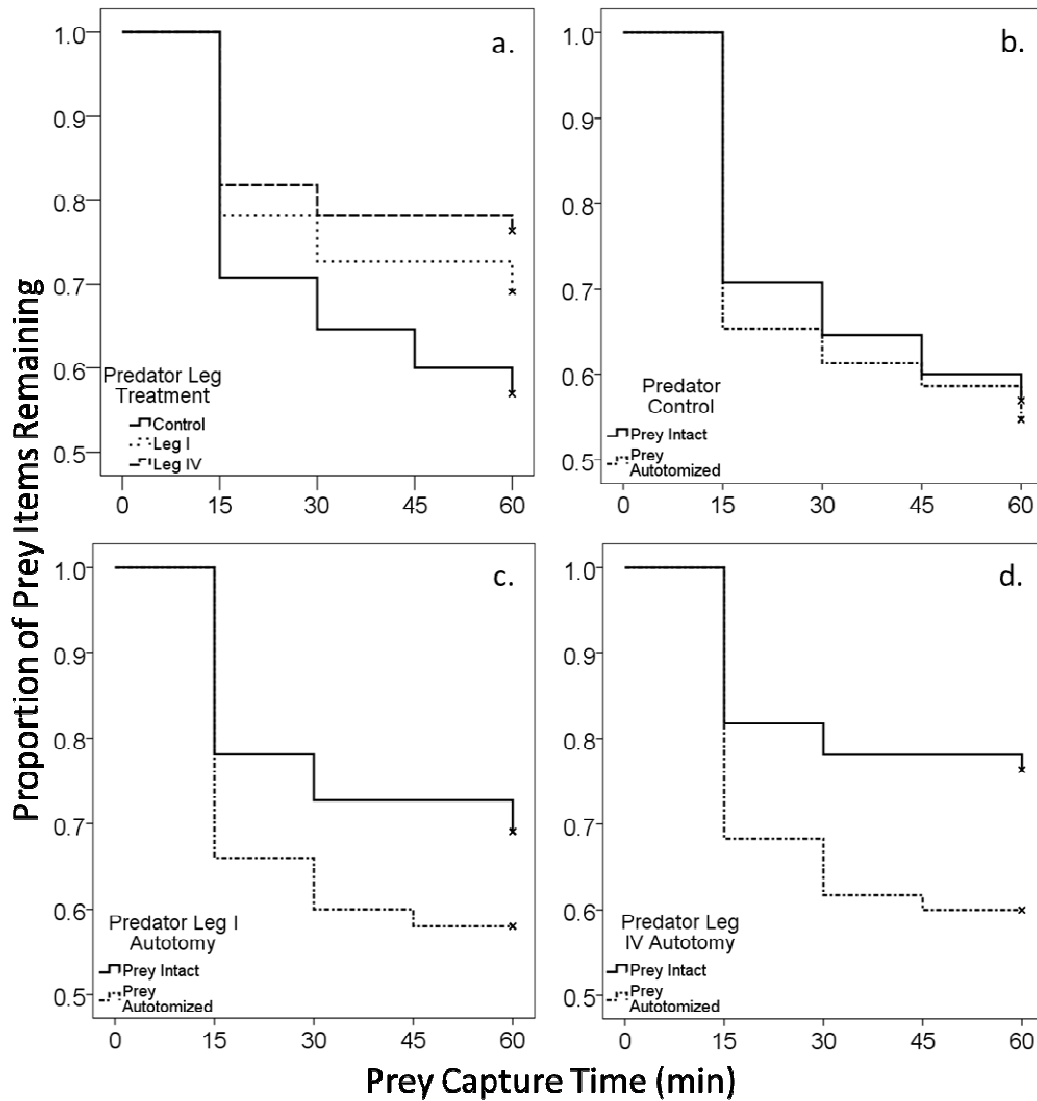
Figure 3.3 Survival plots of the rate of prey survival. In (a) lines represent the rate of prey survival for each predator leg treatment. In (b-d) each graph represents the predator leg treatment (control, leg I autotomy, and leg IV autotomy respectively) comparing survival rates of intact (solid line) and leg IV autotomized (dashed line) prey. Crosses at the end of each survival function denote data that was censored (i.e. at least one prey items in the given group survived the entire 60 minutes).

3.4 Discussion

Approximately 15% of predators captured showed evidence of autotomy. While our sample size was relatively small, this is comparable to previous studies on other

species of wolf spiders, where the frequency of autotomy in field settings has been observed at 16.4% in *Pardosa milvina* (Brueseke et al. 2001) and has been shown to range from 10.9% to 19.3% in *Schizocosa ocreata* (Wrinn and Uetz 2007). Wolf spiders studied here were most commonly found missing legs IV or I, though frequency of autotomy was very similar for all four legs. Wrinn and Uetz (2007) had sufficient sample size (n = 517) to statistically detect differences in leg loss by position in *S. ocreata*, and also found that legs I or IV were the most commonly lost. Other spiders have also been identified as most commonly missing leg IV as well (Holocnemus pluchei; Johnson and Jakob 1999). When considering that autotomy is thought to be a defensive mechanism in the event of an attack, spiders should most commonly autotomize either a leg IV or leg I. If a spider is being attacked, this will most likely elicit a fight or flight response. If individuals flee directly away from their aggressor, their legs IV will be closest to the predator and should be the most frequently autotomized. However, if they are aggressive and/or decide to directly face their attacker, their first pair of legs should be at the greatest risk for attack and subsequent autotomy if the need arises. It is important to note, however, that while predation is most likely responsible for the majority of autotomy events, other factors can also cause spiders to autotomize legs. For example, spiders may autotomize legs during molting when a leg cannot be successfully separated from the molt (Foelix 2010), as a result of intraspecific interactions (Roth and Roth 1984), or in the event of limb injury (Eisner and Camazine 1983).

While there was no significant decrease in running speed following foraging trials, there was a non-significant trend of individuals running slower after having a leg autotomized. Brueseke (2001) also found that wolf spiders missing a leg did not differ from intact individuals in their movement speed, however they measured walking speed as opposed to prompted running speed. Three other studies that did test running speed

(in which the spiders were encouraged to run) found that spiders ran significantly faster with all legs intact than they did following leg autotomy (Amaya et al. 2001, Apontes and Brown 2005, Brown and Formanowicz 2012). While most previous studies support the finding that autotomy of a limb decreases running speed, this metric of locomotive capability may not be a critical factor that influences the success of foraging in wolf spiders. Most wolf spiders attacking prey use short bursts of speed rather than longer duration sprints (Amaya et al. 2001). Therefore, the running speed of a spider may not directly translate into how effective individuals (autotomized or otherwise) are at capturing prey.

While there was no significant effect of predator leg loss on the proportion of prey captured, there was a non-significant trend toward intact predators having greater success, and there were significant differences in the rate of prey survival when prey were intact. Because the number of prey provided was set, the rate of prey survival was the direct inverse of the rate of prey capture. We found that when predators were missing leg IV, prey trended toward higher survival rates than when the predators were intact, that is the rate of prey capture was higher for intact predators versus ones missing leg IV. Wrinn and Uetz (2008) also found that autotomy negatively impacted the rate of prey capture in wolf spiders, but their results only tested the loss of leg I. We did see a trend that missing the first leg appears to detrimentally affect the rate of prey capture compared to controls, however not to the degree that missing a fourth leg does. This is logical, based upon the method of prey capture used by many wolf spiders. The fourth pair of legs are used to leap forward, while the first pair of legs are used to manipulate prey items only after contact with the prey has been made (Rovner 1980). Spiders missing a leg IV may be more likely to have difficulty leaping to attack prey items, and consequently have more failed attacks than intact individuals. Those spiders missing leg

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I may not have trouble with the initial thrust toward prey, but probably have an impaired ability to contain and/or manipulate prey before venom injection and immobilization. Thus, missing a limb or body part responsible for the initial portions of the prey attack phase are likely more detrimental toward predator foraging success than any others.

When both the predator and prey were missing a leg, there were no differences among the predator treatments either in total proportion of prey captured or the rate at which prey items survived. While the two predator autotomy treatments were most likely still affected by the loss of a limb, it appears that this decrease in prey capture efficiency was moderated by the loss of a leg in the prey themselves, a not uncommon event as the prey species used here has been shown to have a field frequency of autotomy between 23% and 41% (C. Brown, D. Formanowicz, and C. Amaya; unpublished data). It has been shown numerous times that organisms that have had a body part autotomized have slower running speeds (including with the prey species used here; Brown and Formanowicz 2012), and thus most likely have a diminished capability to escape from predators (Amaya et al. 2001, Apontes and Brown 2005, Cooper et al. 2009). The decrease in escape speed of the prey items here most likely allowed predators that were impaired to have more success in capturing prey, regardless of the predators' leg loss. Christensen et al. (1996) suggested that the relationship between predator and prey mobility seems to be the most important factor determining the maximal size of prey consumed for aquatic predators and prey. It seems plausible that mobility as it relates to both the predator's ability to capture prey and to the prey's ability to escape attacking predators should translate to a terrestrial environment in much the same way, and that this most likely relates not only to the size of prey consumed, but the number as well.

The habitat heterogeneity relevant to our spider species may contribute to our results as well. It has been suggested that the effect of autotomy on each species is

most likely dependent on their environment and foraging strategies (Amaya et al. 2001, Wrinn and Uetz 2008). Spiders in simple habits that are more mobile and tend to wander more while foraging may be less affected by the loss of a leg (possibly as indicated in our study by no significant effect of leg loss on running speed), than those found in complex environments where ambushing prey may be a more efficient strategy. Many wandering spiders (like wolf spiders) are ambush predators that only attack prey when they come close to the predator (Foelix 2010). In the field, if the predator misses the first attempt at a prey item, it may not get a second chance to capture the escaped prey as a heterogeneous environment may provide refuges for prey to hide (Wrinn and Uetz 2008). The predators used here inhabit a very complex environment of rocky scree, tall vegetation, and leaf litter that accumulates in the creek bed where they forage. While our experimental chambers did include a heterogeneous substrate, it was not as complex as the environment that the predators typically forage in. Additionally, because both the predator and prey were confined to the experimental chambers, if a predator did not capture a prey item on the first attack attempt they would likely be given another opportunity later in the foraging trial. The combination of a less complex habitat in our foraging trials than that of field conditions and the ability of spiders to have multiple opportunities to capture each prey item could have artificially increased the ability of our autotomy impaired predators to capture prey. This could explain the presence of trends in our data suggesting costs of autotomy, but our lack of statistical support.

The long-term consequences of autotomy on foraging success have the potential to significantly alter food web dynamics. For example, keystone predators that exhibit autotomy have been shown to have decreased ability to regulate prey populations (Barrios et al. 2008). However, when intraguild predators and prey are both capable of autotomy, the dynamic between predator success and prey escape can further alter the

mechanics of trophic energy transfer. In fact, some predators will actively seek

autotomized prey items as they are likely to be easier to capture (Bildstein et al. 1989,

Davenport et al. 1992). As seen in our study, the loss of legs in both predator and prey

can potentially mediate the consequences of autotomy on the predators foraging

success, stabilizing the effect of reduced predator foraging on the prey population.

Additional study is essential to fully understand how autotomy at multiple trophic levels

can affect food web structure. Overall while we did find support for the negative effect of

leg loss on foraging, further testing with a larger sample size is necessary to identify if

this trend is significant. However, due to the lack of a detectable cost of autotomy to

foraging in some studies involving simple environments (Johnson and Jakob 1999,

Amaya et al. 2001, Brueseke et al. 2001), but the findings of a negative cost of autotomy

in studies utilizing complex environments (Wrinn and Uetz 2008; current study), we

strongly suggest that future studies investigating the costs of autotomy be conducted in a

testing environment as close to natural conditions as possible.

Chapter 4

The Effect of Food Constraints on Wolf Spider (*Tigrosa helluo*) Fitness

4.1 Introduction

All organisms must allocate their energy reserves towards a variety of different

demands including growth, reproduction, maintenance, and/or defense (Perrin and Sibly

1993, Ahmed et al. 2002, Fedorka et al. 2004). Given that an organism has a limited

amount of energy to invest, the allocation of energy toward one resource demand will

result in less energy to devote to other physiological needs (Stearns 1992, Fedorka et al.

2004). For example, it has been shown that the utilization of defense mechanisms may

result in a trade-off, imposing some cost on other physiological components (Sheldon

and Verhulst 1996, Kraaijeveld and Godfray 1997, Moret and Schmid-Hempel 2000,

Ahmed et al. 2002, Zuk and Stoehr 2002, Sandland and Minchella 2003). According to

optimal defense theory, there should be optimum energy allocation toward defense such

that the benefits of defense outweigh the costs in terms of other fitness-associated traits

(Rigby and Jokela 2000).

Invertebrate pathogen defense is centered on innate immunity, or a set of

disease-resistance mechanisms that function to identify self/non-self molecules (Goldsby

et al. 2000). Broader components of innate immunity include pathogen encapsulation,

antimicrobial defenses, and phagocytosis (Ahtiainen et al. 2006). The prophenoloxidase

(PPO) cascade in particular is considered to be a key component of invertebrate

immunity (Fedorka et al. 2004). The pathway initiates an encapsulation response when

circulating hemocytes (invertebrate blood cells) in the hemolymph (arthropod circulatory

fluid) identify a microbe as non-self, and congregate around the foreign body forming a

capsule (Pathak 1993). The encapsulated pathogens can then be killed by a

combination of isolation from nutrients and the release of cytotoxic molecules secreted by the hemocytes (Armitage et al. 2003, Ahtiainen et al. 2005, Lawniczak et al. 2007).

Melanin is the end-product of the prophenoloxidase cascade in which the enzyme phenoloxidase oxidizes quinone molecules. The reactive quinones act as toxic molecules that kill the pathogen, but also non-enzymatically polymerize into an insoluble melanin barrier (Ahtiainen et al. 2004, Lawniczak et al. 2007). As a result of the prophenoloxidase cascade and reactive quinones, reactive oxygen species (ROS) may also be produced, further damaging pathogens (Moret and Schmid-Hempel 2000). However, ROS can also be produced purposefully with the intent to damage pathogenic cells, rather than simply as a byproduct of the melanin synthesis pathway. This intentional and directed production of ROS to fight pathogens is referred to as a respiratory burst (Nappi and Vass 1998, Ahtiainen et al. 2005). Because ROS can also be damaging to host cells, antioxidants like superoxide dismustase (SOD) can also be produced to enzymatically dismutate the superoxide anions (Nappi and Vass 1998). If invertebrates responding to an immune threat have to allocate additional energy toward the production of active PPO and SOD proteins, they consequently may have less energy to distribute to other physiological processes.

While the activation of the immune response may require that individuals alter their resource allocation pathways, it may only be possible to detect such costs when an animal is energetically stressed. For example, a host may compensate for higher energy expenditure toward immunity by increasing resource intake. This in turn may mask the immune cost, making it difficult to detect energy trade-offs in field captured animals (Moret and Schmid-Hempel 2000, Jacot et al. 2004). Studies are therefore most likely to see evidence of energetic trade-offs between physiological demands when individuals are energetically stressed, such as during periods of starvation. Using the wolf spider

Tigrosa helluo, the goals of this study were to (1) identify how starvation affected

measures of physiological condition and defense, and (2) to ascertain whether starvation

resulted in an energetic trade-off between individual physiological condition and immune

function. I predicted that individuals that were energetically compromised would devote

less energy toward both physiological condition and defense, and that no trade-off

between these physiological processes would be present.

<div align="center">4.2 Methods</div>

4.2.1 Specimen collection and maintenance

Male spiders (*n* = 56) were captured by headlighting at night in Don Misenhimer

Park, Arlington, TX from 12-13 May 2013. Only male spiders were used to mitigate the

effect of excess reproductive energy allocation that using female spiders would present.

Spiders were transported back to the laboratory at the University of Texas at Arlington.

Specimens were housed in sterilized plastic bins and provided with a sand substrate and

a one dram shell vial filled with water and stoppered with a cotton ball for moisture. The

spiders were maintained on a 12:12 light:dark cycle for the duration of the experiment.

Spiders were fed two small crickets every week for two weeks to standardize nutritional

and energetic levels among the individuals. The spiders were then randomly assigned to

four groups: (1) a four week control in which they continued to be fed one prey item each

week, (2) a four week starvation group in which they were not fed for four weeks, or a

corresponding (3) six week control and (4) six week starvation group. Once each set of

treatments had reached their respective four or six week endpoints, spiders had both

measures of fitness and immunity recorded.

4.2.2 Physiological condition data collection

The day before hemolymph extractions, running speed was recorded as a

measure of fitness using a 1 m racetrack (Figure 4.1). The track consisted of tin sheeting

nailed along both sides of a piece of lumber (approximately 10 cm in width). The lumber

was marked in half meter intervals with a small holding chamber adjacent to the

beginning of the track and blocked off with a piece of plexiglass. Spiders were placed in

the holding chamber and allowed to acclimate for approximately five minutes. Once

acclimated, the plexiglass was removed and the spider was prompted to run down the

track with a piece of foam attached to a wooden dowel (Amaya et al. 2001, Brown and

Formanowicz 2012). The running trial was recorded using a digital video camera (Sony

Handycam DCR-SR68). Video editing software (Sony Vegas, v9) was used to determine

the time each individual took to run their fastest half-meter (Brown and Formanowicz

2012). This value then converted to m/s by multiplying by two and then taking the

inverse.

Figure 4.1 The track used to measure spider running speed. The right side of the track
contained a holding chamber (A) for acclimation prior to trials and (B) is the dowel used
prompt individuals to sprint.

Individuals also had a ratio index calculated as a second measure of fitness. A

ratio index is a common measurement of the physiological condition of an individual,

typically calculated as body mass divided by a linear measure of body size (Jakob et al.

1996). In this case, the ratio index was determined by dividing body mass by carapace

length. Each specimen was weighed to the nearest 0.1 mg with a digital scale. Spiders

were then euthanized and preserved with 95% ethanol for microscope analysis. Each

spider then had their carapace lengths measured to the nearest 0.01mm using digital

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microscopy (AmScope-MT, v3.0.0.6) and an image analysis program (Image J, v1.47; National Institutes of Health). After carapace length measurements, each spider has its abdomen dissected to look for the presence of large-scale pathogens such as parasitic worms.

4.2.3 Hemolymph extraction

In preparation for hemolymph extraction, spiders were placed in the refrigerator at 4 ℃ for approximately 30 minutes to limit their mobility and physiological response to anesthetization. The anesthetization chambers consisted of plastic tupperware with a foam insert. A plastic hose was attached to the bottom of the chamber and carbon dioxide (CO_2) gas was pumped into the chamber at approximately 20 psi until the spider was immobilized (Figure 4.2). Using forceps, each leg was gently grasped and forcibly autotomized. The resulting bead of hemolymph at the proximal leg joint was then collected using a micropipette and added to centrifuge tubes containing 200 μL of 50 mM NaPBS (sodium phosphate buffer solution) with a pH of 7.0. This procedure was repeated until all legs were removed. The hemolymph-buffer samples were homogenized and then stored at -80 ℃ to stop prote in activity until ready for immunological assessment.

Figure 4.2 Chamber used for CO_2 anesthetization of spiders. The hosing was attached to a CO_2 tank that controlled the flow of gas into the chamber. Spiders were placed in the chamber with the lid loosely sealed to allow for gas to escape.

4.2.4 Immunity data collection

A suite of fluorometric and colorimetric microassays were optimized and performed in the laboratory to measure constitutive immune activity of spiders from each treatment. These assays measured a variety of immunological factors such as total protein concentration (RED 660® assay), pathogen encapsulation (prophenoloxidase assay) and antioxidant activity (superoxide dismutase assay). All assays were performed with a Synergy 2 spectrometer (Biotek, Winooski, VT).

Total protein concentration was quantified by adding 10 μL of hemolymph sample to 150 μL of RED 660 protein assay reagent (G Biosciences, St. Louis, MO) in a transparent 96-well microliter plate (Greiner Bio-One). A standard curve made from a

serial dilution of 2 mg/ml bovine serum albumin (G Biosciences, St. Louis, MO) was plated and 150 µL of RED 660 protein assay reagent added as well. The standardized concentration curve was calculated using a linear regression formula of known protein concentrations. A static optical density (OD) read of each well was taken at 660 nm immediately after the samples and reagent were combined.

Prophenoloxidase (PPO) activity was acquired by adding 20 µL of hemolymph sample to 10 µL of 50 mM NaPBS pH 7.0 and 10 µL 0.1 mg/ml trypsin (Sigma Aldrich) in a transparent 96-well microliter plate (Greiner Bio-One) and incubated for 30 min at room temperature. To initiate the reaction, 20 µL of 10 mM L-1,3-dihydroxphenylalanine (L-DOPA, Sigma-Aldrich) was added to each well and the OD of the each well was read at 490 nm at 26ºC every 30 s for 25 min. The rate of reaction was determined by calculating the slope during the linear portion of each reaction.

Superoxide dismutase (SOD) activity was measured using the SOD Assay Kit (Sigma-Aldrich). 25 µL of hemolymph sample was added to 200 µL of Dojindo highly water-soluble tetrazolium salt (WST-1; 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-[2,4-disulfophenyl]-2H-tetrazolium, monosodium salt) and 20 µL of xanthine oxidase (enzyme that produces reactive oxygen) in a transparent 96-well microliter plate (Greiner Bio-One) and incubated for 30 min at 37º C. After incubation, the OD of each well was read at 450 nm. SOD activity was quantified by calculating percent reduction of the superoxide ions present at the endpoint of the reaction.

4.2.5 Data manipulation and statistical analysis

All protein concentration values were standardized by dividing them by the carapace length of the spider. Both PPO and SOD were standardized by dividing each value by the standardized protein value for each spider. Scores were identified as outliers if they were more than two standard deviations away from the mean of their

variable distribution. As a result, one value was removed from the six week control running speed data, and one outlier was removed from the six week starvation PPO distribution. To satisfy the assumptions of multivariate normality, protein concentration, PPO, and SOD were natural-log transformed. Independent variables used as either measures of fitness or immunity were analyzed using a 2-way multivariate analysis of variance (MANOVA) with time (either four or six weeks) and starvation treatments as the main effects. To identify if any physiological trade-offs were present, Pearson r correlation analyses were run between all combinations of dependent variables.

4.3 Results

Mortality varied for spiders depending on the treatment. Overall, starvation treatments for both four and six weeks had higher mortality than their respective control groups (four week control, 20%; four week starvation, 25%; six week control, 0%; six week starvation, 58.3%). No parasitic worms were present in any of the dissected abdomens. MANOVA analysis indicated an overall significant effect of starvation, but no statistical effect of time or an interaction between the two main effects (starvation, $F_{5,32} = 3.733$, $P = 0.009$; time, $F_{5,32} = 1.957$, $P = 0.112$, starvation X time, $F_{5,32} = 0.913$, $P = 0.485$; Table 4.1). Due to no significant interaction effect in the MANOVA, univariate analyses of variance (ANOVA) were also examined for each measure of physiological condition or immunity. All univariate ANOVA's were subjected to a Bonferonni correction to avoid inflating Type I error rate.

Table 4.1 MANOVA and univariate ANOVA results for each measure of physiological condition and/or basal immunity and each treatment.

	Time effect			Starvation effect			Time X Starvation		
	F	d.f.	*P*	*F*	d.f.	*P*	*F*	d.f.	*P*
MANOVA									
Wilks' lambda	1.957	5, 32	0.112	3.733	5, 32	**0.009**	0.913	5, 32	0.485
Univariate tests									
Ratio index	1.179	1, 36	0.285	1.652	1, 36	0.207	0.820	1, 36	0.371
Running speed	4.559	1, 36	0.040	5.314	1, 36	0.027	0.979	1, 36	0.329
Protein concentration	0.063	1, 36	0.803	17.091	1, 36	**<0.001**	0.342	1, 36	0.562
PPO activity	2.296	1, 36	0.138	2.022	1, 36	0.164	1.930	1, 36	0.173
SOD activity	0.001	1, 36	0.971	18.006	1, 36	**<0.001**	0.353	1, 36	0.556

Results in bold are significant for the MANOVA at α = 0.05 and at α = 0.01 for the univariate ANOVA's.

4.3.1 Physiological condition

For all variables, there was no time or interaction effect (Table 4.1). Ratio indices were not significantly different between control or starvation groups for either the four or six week treatments ($F_{1,36} = 1.652$, $P = 0.207$; Figure 4.3a). There was however a non-significant trend of six week starved individuals (mean = 0.119 ± 0.031 m/s) having a slower running speed than their corresponding control counterparts (mean = 0.206 ± 0.023 m/s) and individuals from the four week treatments (4 week control mean = 0.230 ± 0.016 m/s, 4 week starvation mean = 0.202 ± 0.016 m/s; $F_{1,36} = 5.314$, $P = 0.027$; Figure 4.3b).
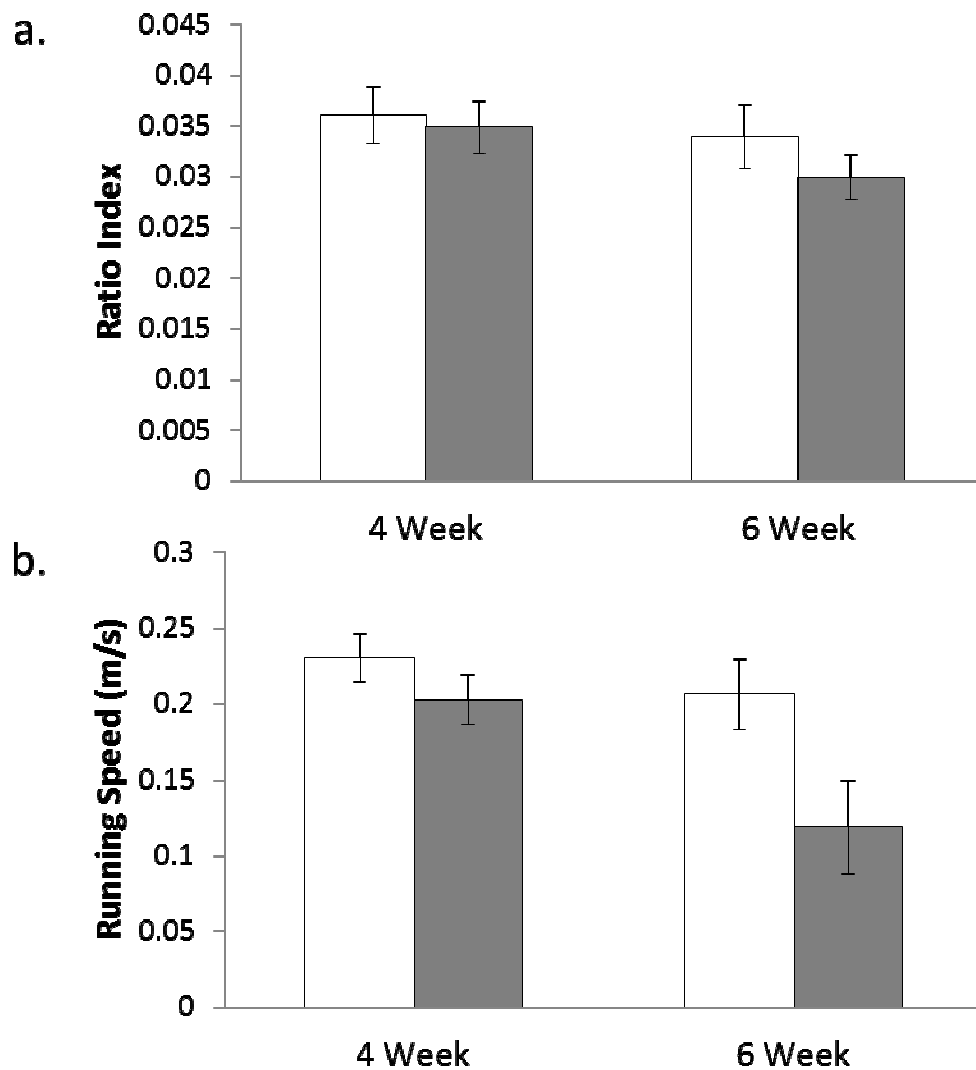
Figure 4.3 Comparison of physiological condition measures between control and starvation groups for both time treatments. White bars and gray bars stand for the control and starvation groups respectively. Error bars represent the standard error of the mean.

4.3.2 Immunity

Individuals that were starved had significantly lower concentration of protein in their hemolymph than control spiders at both four and six weeks (4 week control mean = 0.140 ± 0.029 mg/ml/mm, 4 week starvation mean = 0.042 ± 0.009 mg/ml/mm, 6 week

control mean = 0.183 ± 0.041 mg/ml/mm, 6 week starvation mean = 0.034 ± 0.014

mg/ml/mm; $F_{1,36}$ = 17.091, P < 0.001; Figure 4.4). There were no significant differences

in PPO activity between starvation and control groups, but there was a non-significant

trend at four weeks ($F_{1,36}$ = 2.022, P = 0.164). Four week starvation (mean = 2.088 ±

0.414 activity units) spiders tended to have higher PPO activity than four week control

individuals (mean = 1.150 ± 0.156 activity units). This trend was not present for the six

week spiders (Figure 4.5a). Lastly, SOD activity was significantly higher for both the four

and six week starvation groups than their respective controls (4 week control mean =

1202.298 ± 345.537 activity units, 4 week starvation mean = 2657.179 ± 415.711 activity

units, 6 week control mean = 913.230 ± 218.672 activity units, 6 week starvation mean =

3579.134 ± 837.280 activity units; $F_{1,36}$ = 18.006, P < 0.001; Figure 4.5b).
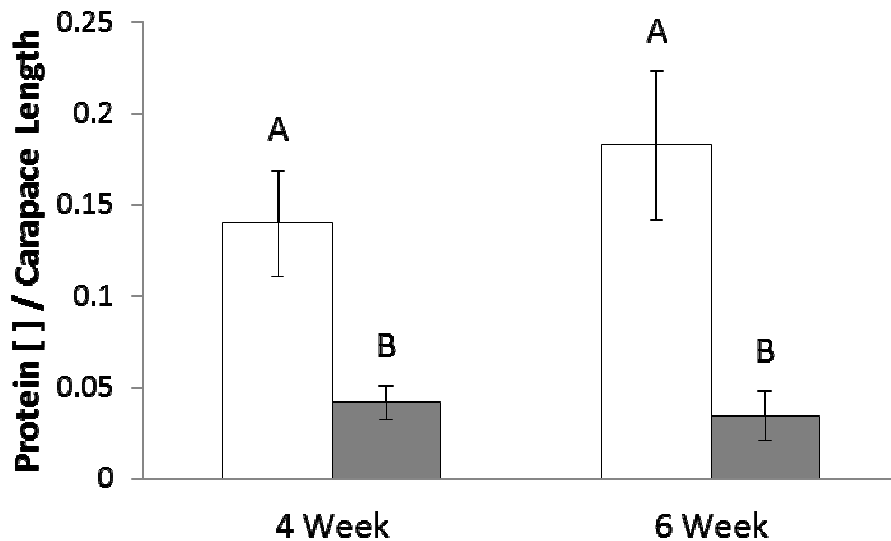
Figure 4.4 Comparisons of the carapace length corrected protein concentration for
starvation treatments for both the four and six week groups. White bars represent control
groups and gray bars represent starvation groups. Error bars indicate the standard error
of the mean. Letters above the bars designate significant differences.
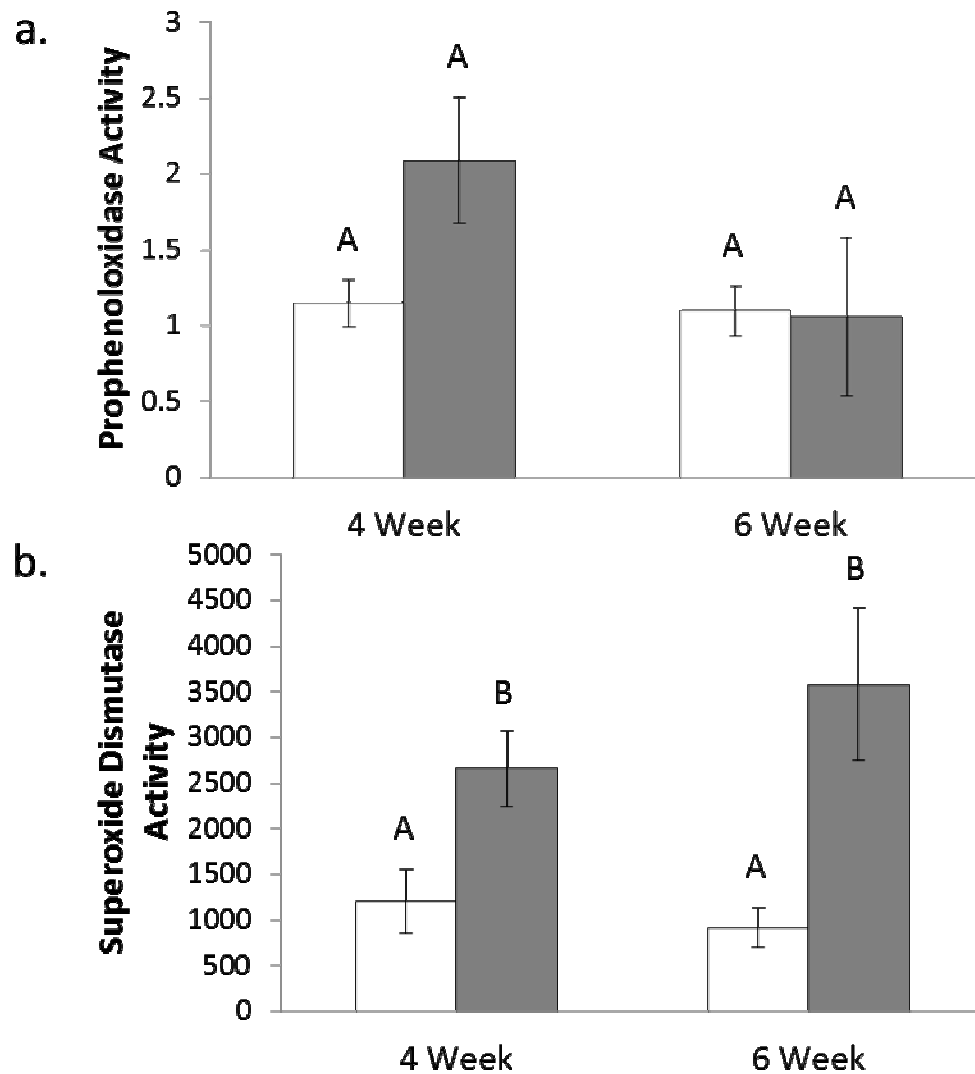
Figure 4.5 Comparisons of immunity measures the two starvation groups for both of the time treatments. White and gray bars represent the control and starvation groups respectively, and error bars represent the standard error of the means. Letter above the bars denote significant differences between the groups.

4.3.3 Trade-offs

Pearson's *r* correlations identified a number of significant relationships between dependent variables. Protein concentration was moderately correlated with both ratio indices and running speed (ratio index, *r* = 0.331, *P* = 0.032; running speed, *r* = 0.378, *P*

= 0.015). However, protein concentration demonstrated a moderate negative relationship

with PPO activity (r = -0.573, P < 0.001) and a very strong negative relationship with SOD

activity(r = -0.978, P < 0.001). In turn, SOD was also moderately correlated with running

speed (r = 0.382, P = 0.014). Lastly, SOD and PPO activity were moderately correlated

with one another (r = 0.584, P < 0.001). All other pairwise correlations were not

significant (Table 4.2).

Table 4.2 Correlation matrix of all combinations of variables for the four week starvation spiders. The top value for each correlation represents the correlation coefficient and the bottom number corresponds to the P-value. Values in bold are significant at the α = 0.05 level.

	Ratio Index	Running Speed	Protein [ ]	SOD Activity
Running Speed	0.127			
	0.428			
Protein [ ]	**0.331**	**0.378**		
	0.032	**0.015**		
SOD Activity	-0.276	**-0.382**	**-0.978**	
	0.077	**0.014**	**<0.001**	
PPO Activity	0.136	-0.114	**-0.573**	**0.584**
	0.396	0.484	**<0.001**	**<0.001**

4.4 Discussion

Energetic stressors have the potential to alter energy allocation pathways,

resulting in trade-offs among physiological processes. By exposing organisms to

potentially common environmental stressors like starvation that are typical for many

animal populations, we can gain insights into the energetic decisions that such organisms

face in the field (Moret and Schmid-Hempel 2000). I predicted that energetically stressed

organisms would devote less energy toward both physiological condition and immune

parameters. We also did not expect to see the presence of a trade-off between

physiological condition and defense, predicting that organisms that were energetically

compromised would continue to allocate energy toward physiological patterns in similar

ways to those that were not compromised.

We did not detect any difference among ratio indices (body mass/carapace length) between control and starved spiders. It is possible that a hydration effect negated the effect of starvation. Spiders obtain the majority of their water from the consumption of prey fluids (Vollmer and MacMahon 1974, Butt and Taylor 1995). Given that over 75% of male wolf spider mass is water, spiders that were starved may have maintained their body mass through water consumption (Carrel 1990). Because carapace length should not change appreciably as a result of starvation, and supplemental water could maintain body mass loss due to dehydration, ratio indices may have fluctuated very little between treatments.

As we expected, there was a trend that running speed was slower in spiders that were starved for six weeks. This makes sense given the context that starved spiders also had significantly lower protein concentration in their hemolymph. Invertebrate prey (such as the crickets provided as a food source here) have been shown to be very high in protein, and the majority of the diet of many predaceous arthropods consists of the protein from their prey items (Bowen et al. 1995). These proteins can then be catabolized for energy and used for a variety of metabolic or physiological activities (Yurkowski and Tabachek 1979). Since spiders that were starved had no access to prey items, they would also have had limited protein resources. Consequently, starved individuals would have had less energy to devote to activities like running speed. Another possible explanation for lowered running speed is that the spider may be actively conserving energy. It has been shown that one of the possible mechanisms to deal with energetic limitations imposed by starvation may involve reductions in energy expenditure (McCue 2010). It is therefore most likely a combination of a choice to limit energy usage and a stressed energy budget due to starvation that decreased running speeds.

Overall, our data show that PPO and SOD activity is higher among individuals that are starved than those that are being fed regularly. Our results here also conflict with several terrestrial invertebrate studies that showed a lower degree of immunocompetence when organisms were nutritionally and energetically deprived (Suwanchaichinda and Paskewitz 1998, Harshman and Zera 2007). There are however several potential explanations for these somewhat counterintuitive results. It may simply be that starved spiders were upregulating their immune defense. However, given that investment in an immune response has been shown numerous times to be physiologically demanding (Moret and Schmid-Hempel 2000, Jacot et al. 2004, Harshman and Zera 2007), and that starved individuals were energetically stressed already, this may not be the case.

An alternative explanation is that PPO and SOD activity is artificially inflated in spiders that were starved. Both PPO and SOD measured here are expressed as a proportion of the total protein content in each sample. Under starvation conditions, some invertebrates will attempt to reduce their rate of protein synthesis, and extend the half-life of their existing cellular proteins (Anchordoguy et al. 1993, Hand and Hardewig 1996). However, basal metabolic activity must still go on, which will use up the storage metabolic proteins and catabolize other proteins for energy (Clarke 1980, Hewitt 1992). The combination of attempting to extend the half-life of their cellular protein pool, but continuing to deplete proteins not involved in immune defense either through metabolism or catabolism could result in immune proteins like PPO and SOD being conserved. Thus, the proportion of PPO or SOD present against the total protein concentration may be artificially inflated.

The increase in SOD seen in our starved spiders could also be due to the overproduction of reactive oxygen species (ROS). Under normal conditions, ROS is

produced at low levels continuously by metabolic processes (Møller 2001, Apel and Hirt 2004, Suzuki and Mittler 2006). However, the production of ROS is also an important component of the general stress response (Tiwari et al. 2002, Lesser 2006, Dowling and Simmons 2009). The imposition of abiotic and biotic stressors can dramatically elevate ROS production (Mittler 2002, Turrens 2003, Bhattacharjee 2005, Suzuki and Mittler 2006, Miller et al. 2008, Gill and Tuteja 2010). In fact, starvation and nutrient deprivation have been linked to increased ROS production (Deng et al. 2002, Mittler 2002). Because ROS can damage a variety of vital macromolecules and tissues, organisms must also produce additional antioxidants (like SOD) in response to bind to ROS and dismutate the superoxide radicals (Nappi and Vass 1998, Møller 2001, Apel and Hirt 2004). Elevated ROS activity (and consequently SOD activity) could also be explained by increased activity of the innate immune defense. If PPO is being upregulated for immune defense, ROS are produced as a byproduct of the metabolic activity required to synthesize such proteins. Lastly, ROS production may be increased intentionally by way of respiratory bursts, again used to further damage pathogens (Sorci and Faivre 2009).

Overall, we found support for a trade-off between physiological condition and immunity, as we did have a significant negative correlation between running speed and SOD. This agrees with most literature in the field of ecoimmunity that there should be substantial energetic costs to the activation of the immune defense, resulting in less energy to devote to other physiological considerations (Moret and Schmid-Hempel 2000, Jacot et al. 2004, Ahtiainen et al. 2005, Harshman and Zera 2007). However, we also found that energetically compromised individuals appeared to sacrifice running speed to allocate more energy toward immune parameters. It could be that starved individuals were more susceptible to infection by pathogens and so they counteracted this by altering their resources allocation pathways. However, this is difficult to determine as we

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have no data to date on whether our spiders were under immune threat by microbial pathogens or not. We therefore suggest that future studies involved in immune defense energetic allocation should integrate methods to identify the degree of individual potential virulence factors. Because the cost of immune defense can be so high, further study is necessary to quantify proximate and ultimate costs of immune activation.

Chapter 5

Concluding Remarks

This dissertation has shown that there are a variety of factors that can potentially influence how strong of a pressure predators can exert on prey populations through direct consumption. In chapter 2, I found that sexually dimorphic differences in prey capture ability in *T. helluo* were primarily driven by female biased morphology and differences in trophic characteristics. Females were able to subdue more prey, and do so faster than conspecific males. I also saw evidence of behavioral dimorphism between the sexes. Females were generally more aggressive than males and were more likely to spend more time manipulating prey items, and less time moving than males. Because of these behavioral differences, I suggest that male and female wolf spiders have different search behaviors. The combination of morphological and behavioral dimorphisms ultimately resulted in females capturing a significantly higher proportion of prey items at higher prey densities (Figure 5.1a-b).

In chapter 3, I found little evidence that impaired body condition in the context of autotomy affecting prey capture. I found that spiders missing legs did not have significantly reduced running speeds. The data also suggested that individuals missing legs capture a lower proportion of prey items than those that have all legs intact, and that spiders missing legs capture prey at a slower rate than intact spiders as well. Predators that were missing a leg IV tended to be negatively the most in their ability to capture prey. Limb autotomy most likely impairs the ability of spiders to subdue their prey, leading to decreases in prey consumption, and ultimately decreasing the strength of direct consumption on prey populations (Figure 5.1c).

Lastly, in chapter 4 I investigated how energetic stress could lead to physiological condition and defense trade-offs that could potentially affect spiders' ability to reduce prey population sizes. Spiders that were starved tended to have reduced running speeds, but appeared to allocate more energy toward immune defense, resulting in an apparent energetic trade-off. I postulate that energetic stress impedes the completion of a predation sequence by decreasing running speed, making starved spiders less likely to be competitive at pursing prey items (Figure 5.1d).
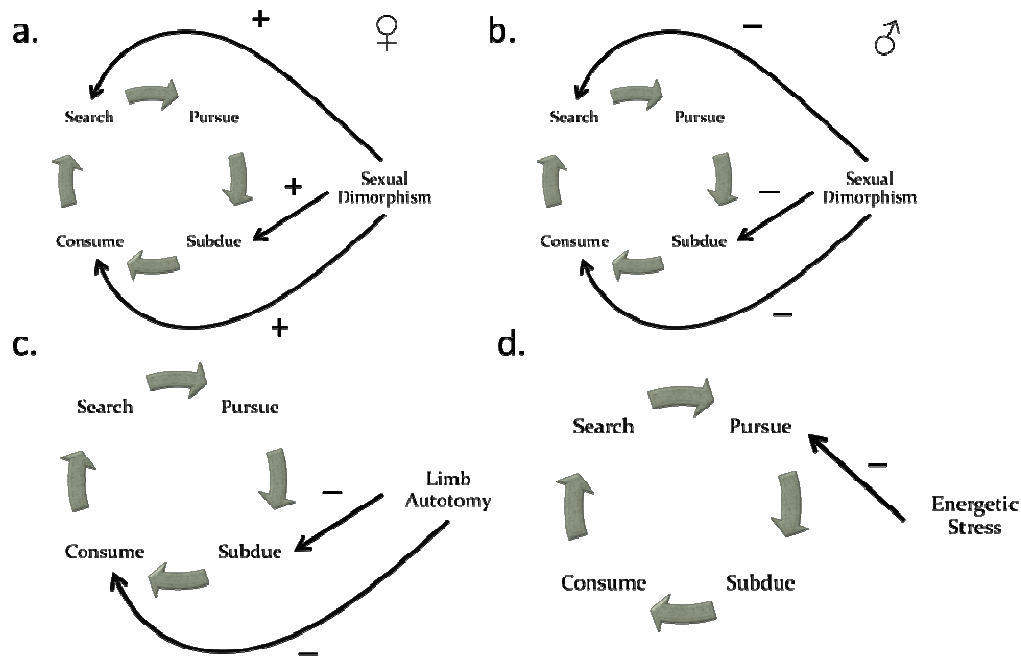
Figure 5.1 Conceptual diagrams of predation sequences. Arrows indicate the direction of impact of (a) sexual dimorphism (female), (b) sexual dimorphism (male), (c) limb autotomy, and (d) energetic stress, on the appropriate portion of the sequence.

Overall, data generated from this dissertation provide evidence that a variety of factors can impair the ability of cursorial hunting spiders' ability to suppress prey populations through direct consumption. Few conceptual food webs focus on sex ratios or the body state of individuals within a food web. The data here illustrate that such

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factors may be important in identifying the strength of the predation on lower trophic levels, ultimately playing a part in dictating community structure. By integrating this data into food web models, we may begin to further understand the dynamics behind how predator-prey interactions function to structure communities.

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Biographical Information

Matthew M. Steffenson was born in Schenectady, New York to Elizabeth and Michael Steffenson, on August 3, 1984. He graduated from Guilderland Central High School in 2002. Matthew graduate with a Bachelors of Science from Siena College in 2006. After taking a year off, he then continued his education at Tennessee Technological University in the laboratory of Dr. Chris Brown, graduating with a Master's degree of Science in Biology in 2009. He began his degree at the University of Texas at Arlington in 2009, and graduated with a PhD in Quantitative Biology under the supervision of Dr. Laura D. Mydlarz in 2014. His research interests are based in life-history and behavioral ecology, with a recent foray into ecoimmunity. Matthew has conducted fieldwork in Arizona, New Mexico, and Texas. He has two peer-reviewed publications published or accepted, and has been the awarded grants from the American Arachnological Society, the American Museum of Natural History, and the Phi Sigma Society. He has received two awards for presentations at the Annual Celebration of Excellence by Students at the University of Texas at Arlington, and was the recipient of the T.E. Kennerly Award for Excellence in Teaching from the Biology Department, also at the University of Texas at Arlington. Matthew has accepted a tenure-track assistant professor position at Adams State University in Alamosa, Colorado starting in August 2014.