A New Perspective on Giving-Up Density Experiments and the Landscape of Fear

Jordan D McMahon

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A new perspective on giving-up density experiments and the landscape of fear

By

Jordan D. McMahon

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Biology
in the College of Arts and Sciences

Mississippi State, Mississippi

May 2018
A new perspective on giving-up density experiments and the landscape of fear

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Non-consumptive effects that predators have on prey are important to ecosystems. The perceived risk of predation can alter feeding behavior. Giving-up density (GUD) experiments have been a foundational method to evaluate perceived predation risk, but rely on the assumption that food preferences are absolute. However, nutritional preferences are context dependent and can change with risk. In my first chapter, I used spiders and grasshoppers to test the hypothesis that covariance in nutritional preferences and risk may confound the interpretation of GUD experiments. My results demonstrate that predation risk and nutritional preferences covary and can confound interpretation of GUD experiments. In my second chapter, I use a behavioral observation experiment to further explore non-consumptive effects, as well as the movement of prey in response to predation risk.
ACKNOWLEDGEMENTS

I would like to thank my family for support and inspiration, as well as help with field work over the past two years. I would also like to thank M.S. Siegle-Gaither for field and experimental help. Additionally, I thank C.J. Speights for statistical consultation, and Dr. Mark Hunter for inspiration. A special appreciation goes to my committee, for much help with field site acquisition, statistical consultation, and guidance along the way.
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CHAPTER I
COVARIANCE BETWEEN PREDATION RISK AND NUTRITIONAL
PREFERENCES CONFOUND INTERPRETATIONS OF GIVING-UP DENSITY
EXPERIMENTS

1.1 Introduction

Predators affect ecosystems by having direct effects on prey that cascade down to lower trophic levels, and these effects can arise in two ways (Paine 1980, Hunter and Price 1992, Polis et al. 2000, Peckarsky et al. 2008). First, predators can have consumptive effects by killing prey and reducing their population abundance (Schmitz et al. 1997, Lima 1998a). Second, predators can have non-consumptive effects that arise from the prey’s response to the risk of predation (Schmitz et al. 2004, Preisser et al. 2005, Trussell et al. 2006, Peckarsky et al. 2008). Non-consumptive effects manifest in a myriad of ways and can have broad impacts on prey, including their community- and ecosystem-level interactions (Brown and Kotler 2004, Peckarsky et al. 2008, Hawlena and Schmitz 2010a, b, Schmitz et al. 2010, McCauley et al. 2011, Hawlena et al. 2012, Hermann and Landis 2017). Thus, understanding how prey perceive risk and quantifying the context dependency of their responses has furthered our holistic understanding of predators as both consumptive and non-consumptive forces in ecosystems.

One of the most common approaches used to measure and compare predation risk among different areas are giving-up density (GUD) experiments (Brown 1988, Brown
and Kotler 2004, Bedoya-Perez et al. 2013). These experiments use a depletable food resource to estimate perceived predation. In general, dishes containing a food and inedible substrate are placed in different areas for a standardized amount of time, then the amount of unconsumed food is quantified. The amount of food at which prey stop using the resource patch is assumed to indicate the point at which the benefits of foraging on the food no longer outweigh the costs, and is known as the giving-up density (Brown 1988). While the GUD framework incorporates three main costs of foraging, including the energetic costs of foraging and missed opportunity costs of foraging in another patch, GUD experiments are most often used to assess the third cost, perceived predation risk (Verdolin 2006, Bedoya-Perez et al. 2013). While the risk of predation can be measured independently, the perception of risk for a prey item cannot be measured directly, so GUD experiments provide a useful proxy where perceived predation risk is presumed greater as more food is left unconsumed (Brown 1988, Bedoya-Perez et al. 2013).

Implicit in the GUD framework is the assumption that the prey’s evaluation of the food is consistent among sites, so that any change in GUD can be attributed to differences in foraging costs such as perceived predation risk among sites (Brown 1988). In other words, GUD experiments assume that the perceived value of the food item does not covary with predation risk (Leaver and Daly 2003). However, emerging literature on the effects of predation risk on prey diet selection challenge this assumption (Hawlena and Schmitz 2010a). Further, some studies suggest that predators are capable of causing prey to selectively forage on certain types of foods (Hawlena and Schmitz 2010b). Among the most well-studied examples comes from an arthropod system, where predation risk from spiders causes grasshoppers to shift their diet and increase
consumption of carbohydrate-rich plants (Beckerman et al. 1997, Schmitz 1998, Barton 2010). The mechanism causing this diet switch has been tested explicitly by using artificial diets that differed only in carbohydrate and protein content (Hawlena and Schmitz 2010a, Schmitz et al. 2016). In these studies, predation risk elevated grasshopper metabolism and increased energy demands, which grasshoppers met by preferentially consuming carbohydrate-rich food relative to protein-rich food. While there is some evidence that temperature may also affect preference for carbohydrates and protein by elevating metabolism (Schmitz et al. 2016), most studies have found that dietary preferences of grasshoppers are invariant across temperatures and driven entirely by predation risk (Barton and Schmitz 2008; Barton et al. 2009; Barton 2010; Barton 2011). Thus, predation risk and nutritional preference may not be independent, as consumption of the different food types (i.e., carbohydrate-rich or protein-rich) can covary with risk.

Covariance between predation risk and nutritional preference may confound the interpretation of GUD experiments. For instance, if predation risk increases the perceived value of carbohydrates, GUD experiments using carbohydrate-rich food may underestimate predation risk because the prey increasingly seeks out carbohydrate-rich food (Hawlena and Schmitz 2010a) and reduce it to a lower than expected GUD. Conversely, because protein-rich foods may be devalued with increasing risk, GUD experiments using foods high in protein may overestimate predation risk, because prey give up on the resource at higher GUDs than expected. Given the wide-use of GUD experiments, as well as their foundational role in understanding non-consumptive effects of predators (Bedoya-Perez et al. 2013), resolving whether covariance in predation risk...
and food preference confounds interpretation of this common method is important to predator-prey ecology.

I tested the hypothesis that covariance between predation risk and prey nutritional preferences can confound the interpretation of GUD experiments using spiders and grasshoppers. First, I tested if predation risk altered grasshopper preference for carbohydrate-rich or protein-rich foods in choice and no-choice experiments. I then compared the consumption and giving-up densities of carbohydrate-rich and protein-rich foods under a gradient of known predation risk (i.e., spider density) in a mesocosm experiment. I predicted that if food preference covaried with predation risk, the effect of predation risk on GUD would differ between food types, confounding the estimation of predation risk from GUD experiments.

1.2 Methods

I conducted a series of experiments in July 2016 to evaluate the covariance of predation risk and diet choice, and to determine implications for GUD experiments. Foraging behavior of 3rd instar Melanoplus femurrubrum grasshoppers was studied (Orthoptera: Acrididae), as a function of predation risk from a sympatric spider predator Tibellus oblongus (Araneae: Philodromidae). While others have characterized T. oblongus as a “sit and pursue” predator (Miller et al. 2014), my observations of this predator are consistent with a “sit and wait” (ambush) hunting mode. All arthropods were wild-caught from field sites near Starkville, MS, USA. Ambush predators are known to alter M. femurrubrum behavior and activity patterns (Miller et al. 2014), and cause them to shift their microhabitats. Additionally, at least one spider species (Pisaurina mira)
induces a diet shift towards more carbohydrates and less protein (Schmitz et al. 1997, Barton 2010, Hawlena and Schmitz 2010a).

Following established protocol (Lee et al. 2002, Warbrick-Smith et al. 2006, Behmer and Joern 2008, Hawlena and Schmitz 2010a), I created two artificial diets that differed only in their proportion of carbohydrates and protein. The protein-rich diet contained 28% protein and 7% carbohydrate by mass (hereafter referred to as P-rich), and the carbohydrate-rich diet contained 28% carbohydrate and 7% protein by mass (hereafter referred to as C-rich). Specific ingredients and protocol for making artificial diets are available in Appendix A.

1.2.1 Choice and no-choice experiments

I tested the hypothesis that predation risk covaried with grasshopper consumption of C-rich and P-rich foods using a factorial design that crossed food type (C-rich only, P-rich only, or both) and predation risk (presence of absence of a T. oblongus spider). To confirm that grasshoppers would choose a carbohydrate-rich food source over a protein-rich food source, a choice design was used. Additionally, since GUD experiments often present just one food type, I used a complementary no-choice design to test my hypothesis that predation risk affected the consumption of each food type differently. The experimental units were commercially available plastic terraria (23.2 x 16.8 x 15.24 cm; Lee’s Pet Products, San Marcos, CA), each containing three small, uncovered Petri dishes (two for food, one for water; 35 x 10 mm). In choice experiments, grasshoppers were presented with two dishes, one for each food type (C-rich or P-rich). In no-choice experiments, grasshoppers were presented with two dishes containing the same food type (i.e., C-rich or P-rich). Additionally, each terrarium contained another Petri dish (100 x
15 mm) which held a spider (risk treatments) or was left empty in control (no predation risk) treatments. To facilitate transmission of spider cues (e.g., odors), 15 holes (3mm diameter) were drilled into the lid of each Petri dish. In risk treatments, spiders were provided with water in their Petri dishes. This experiment was conducted on benchtops in a laboratory under ambient light and room temperatures (approximately 21°C). Terraria were arranged linearly in seven blocks within the same laboratory, with opaque partitions between each terrarium. In addition to each of the six possible treatment combinations of the factorial design, one arthropod-free terrarium per block containing only food and water was included. The additional terraria allowed for quantification of changes in food weight not due to grasshopper consumption (e.g., humidity changes) and correct for these changes in my final analysis (Schmitz et al. 2016).

The day before beginning the experiment, arthropods were collected from the field and allowed to acclimate to laboratory conditions for approximately 24 hours. I randomly assigned terraria to treatments, then placed 100 mg of food into each food dish according to treatment assignments (C-rich only, P-rich only, or both), and approximately 3 ml of water into each water dish. A single adult spider was then stocked into the large Petri dish within terraria assigned as predation risk treatments. Finally, the experiment was initiated by releasing a single 3rd instar grasshopper into each terrarium (excluding the arthropod-free terraria).

Each trial was terminated after three days to avoid any long-term effect of persistent exposure to a high level of perceived risk. Total consumption was then calculated by subtracting final weight from initial weight. Using data from the arthropod-free terraria, moisture loss/gain during each trial was corrected for, and mean daily
consumption rates of grasshoppers for each food type was calculated. In the event of a grasshopper death, the data was discarded. This experimental design was repeated four times using fresh arthropods, food, and water during each trial. To analyze the effects of predation risk on grasshopper consumption two separate linear models were used, one for choice experiments (terraria containing both food types) and the second for no-choice experiments (C-rich only versus P-rich only). Food type and spider presence were treated as fixed effects, and block was treated as a random effect. All analyses were conducted using the statistical computing language R (R Core Team 2017).

1.2.2 Giving-up density experiment

The hypothesis that covariance in predation risk and nutritional preferences can confound the interpretation of GUD experiments was tested using a factorial design that crossed food type (C-rich or P-rich) and six levels of predation risk (0, 1, 2, 3, 4, or 5 spiders). The hypothesis that increasing predator density increased the risk of predation was also tested by measuring grasshopper mortality throughout the experiment. Six mesh enclosures were used as experimental units (Bug Dorm-2, BioQuip, Rancho Dominguez, CA, USA; 60 x 60 x 60 cm) and randomly assigned to treatments in a factorial design crossing spider abundance (0, 1, 2, 3, 4 or 5 spiders) and food type (P-rich or C-rich). To simulate giving-up density experimental conditions, I created a resource patch with diminishing returns (Brown 1988) by adding an inedible substrate (150 4.4 mm stainless-steel ball bearings) to each food dish. Each enclosure also had a dish (100 x 15 mm) containing water, and perching sites for grasshoppers (nine green pipe cleaners inserted vertically into a foam substrate). This experiment was conducted on benchtops in a laboratory under ambient light and room temperatures (approximately 21°C). Enclosures
were arranged in two rows of three enclosures each with opaque partitions between them. To account for changes in food weight not due to grasshopper consumption (e.g., humidity changes), each block also had reference dishes which contained 1,000 mg of either C-rich and P-rich foods within the inedible substrate.

The day before starting each trial, arthropods were collected from the field and allowed to acclimate to laboratory conditions for 24 hours. Each enclosure was randomly assigned to a treatment, before receiving 1,000 mg of food to a Petri dish containing inedible substrate, as well as a dish containing ~15 ml of water. The experiment was initiated by releasing the designated number of spiders according to treatment assignments and eight to ten grasshoppers into each enclosure. Multiple grasshoppers were used to simulate GUD conditions (e.g. multiple foragers in the wild).

Due to limitations in space, enclosures, and study organisms, the experiment was conducted in three trials on different days, lasting three to five days each. The first trial included risk treatments with 0, 2, and 5 spiders. trial 2 included risk treatments with 0, 1, and 4 spiders. trial 3 included risk treatments with 0, 2, and 3 spiders. Using the food dishes left out of the enclosures, moisture loss/gain was corrected for during each trial based on the percentage change in mass. To account for different densities of grasshoppers among trials due to mortality (consumptive and non-consumptive), and to examine the effect of predator density on grasshopper mortality, the number of dead grasshoppers was recorded each day.

The hypothesis that covariance between predation risk and nutritional preferences confound giving-up density experiments was tested by comparing the effects of increasing predator density on GUD (i.e., weight of unconsumed food) for both food
types. To do so, a linear model with the amount of unconsumed food (GUD) as the response variable was used, and predator density and food type were treated as fixed effects. To explicitly test whether increasing predation risk affected the GUD of each food type, separate linear models were run for each food type with giving-up density as the response variable, and predator density as a fixed effect. To test the hypothesis that predator density and nutritional preference covary, consumption results were standardized across trials that had different numbers of grasshoppers and duration. This was accomplished by dividing total consumption (mg) by the average number of grasshoppers alive during the trial, and number of days that each trial lasted. The hypothesis was then tested by using a linear model with average consumption/grasshopper/day (mg) as the response variable, and with predator density and food type treated as fixed effects. To explicitly test whether increasing predation risk affected consumption of each food type differently, separate linear models were run for each food type with average consumption/grasshopper/day (mg) as the response variable, and with predator density treated as a fixed effect. To confirm that predator density affected predation risk, a linear model with number of grasshoppers that died as the response variable, and with predator density and food type treated as fixed effects were used. All analyses were conducted using the statistical computing language R (R Core Team 2017).

1.3 Results

1.3.1 Choice and no-choice experiments

I found a significant interactive effect of predation risk and food type on consumption when grasshoppers were simultaneously presented with both food types
(choice experiment: \( F = 15.59, d.f. = 27, P < 0.001 \)). Grasshoppers in the choice experiment increased their consumption of the C-rich food and decreased consumption of the P-rich food when exposed to predation risk (Fig. 1.1a). I found a similar pattern in the no-choice experiment, where there was also a significant interactive effect of predation risk and food type on consumption \((F = 15.43, d.f. = 24, P < 0.001)\). Grasshoppers presented with only P-rich food consumed less food while experiencing predation risk, whereas grasshoppers presented with only C-rich food consumed more food with risk (Fig. 1.1b). Additionally, differences in consumption were not explained by block. A complete summary of statistical results for choice and no-choice experiments are displayed in Table 1.1.

### 1.3.2 Giving-up density experiment

I found a significant interactive effect of predator density and food type on giving-up density \((F = 7.98, d.f. = 14, P = 0.01)\). Increasing predator density increased GUD when using the P-rich food \((F = 5.767, d.f. = 7, P = 0.047)\) but there was not a significant effect when using the C-rich food \((F = 2.210, d.f. = 7, P = 0.18; \text{Fig. 1.2a).} \) Similarly, I found a significant interactive effect of predator density and food type on consumption \((F = 9.35, d.f. = 14, P = 0.009)\). Increasing predator density decreased average consumption of P-rich food \((F = 7.07, d.f. = 7, P = 0.03)\), but there was no significant effect when using C-rich food \((F = 2.39, d.f. = 7, P = 0.17; \text{Fig. 1.2b})\). Grasshopper mortality increased with predator density \((F = 5.96, d.f. = 14, P = 0.03)\), but mortality was not affected by food type or a food type * predator density interaction. A
complete summary of statistical results for the giving-up density experiment is displayed in Table 1.2.

1.4 Discussion

The mesocosm experiments allowed me to manipulate levels of predation risk and measure the resulting giving-up density (GUD). However, these types of experiments are generally conducted by measuring GUD to estimate unknown predation risk (Bedoya-Perez et al. 2013). Accordingly, if results from the GUD experiment were used to estimate predation risk within the mesocosms, the results using protein-rich food would be as expected: a positive relationship between GUD and predator density (Fig. 1.2a). That is, increasing predator density increased costs of foraging and the prey consumed less of the food. However, when using a carbohydrate-rich food, increasing predator density had no effect on food consumption, and the analysis of the average consumption per grasshopper per day revealed the same trend (Fig. 1.2b). Thus, results when using C-rich food suggest that predation risk is the same whether or not spiders are present, which is fundamentally untrue: no spiders are less risky than some spiders, as demonstrated by the positive relationship between spider density and grasshopper mortality in this experiment. Thus, my experiments not only demonstrate that food type can change the results of GUD experiments, but that using GUD experiments to evaluate predation risk may be misleading because prey nutritional preferences covary with predation risk.

While these results have surprising implications for GUD experiments, they are consistent with emerging theory about non-consumptive effects of predators on prey diet. The geometric framework of nutrition predicts that intake targets can differ across life
stages (Simpson and Raubenheimer 1993), among different sexes (Simpson and Raubenheimer 2007), and depending on the presence or absence of toxins in some food items (Behmer et al. 2002). These targets can also change as a function of different environmental conditions (Raubenheimer et al. 2009, Schmitz et al. 2016), so it is not surprising that grasshoppers shift their diet in the presence of predators (Beckerman et al. 1997, Schmitz and Suttle 2001, Barton et al. 2009). Recent laboratory studies have demonstrated that the shift occurs because predation risk increases grasshopper preference for carbohydrate-rich foods (Hawlena and Schmitz 2010a, Schmitz et al. 2016). Consistent with this previous work, results from choice experiments revealed that predation risk caused grasshoppers to increase consumption of C-rich food and decrease consumption of P-rich food when both food types were present (Fig. 1.1a). The no-choice experiment revealed a similar pattern, demonstrating that even when an alternative food is not present, predation risk caused grasshoppers to reduce consumption of P-rich food and increased their consumption of C-rich food (Fig. 1.1b). Each of these three experiments (choice, no-choice, and GUD experiments) revealed similar patterns, corroborating my hypothesis that covariance between predation risk and nutritional preferences can confound the interpretation of GUD experiments.

However, not all GUD experiments necessarily suffer from confounded experimental designs. There are two general approaches used in GUD experiments: within-site or among-site experiments. Within-site GUD experiments evaluate the effect of a factor (e.g., presence of predator urine) on GUDs at a common site. That is, the same individuals are potentially foraging on foods in both treatments (e.g., predator urine present or predator urine absent). By including both treatments within the same site, prey
have experienced the same level of predation risk, and are likely to have similar nutritional preferences. So, that approach may not be confounded by fear-mediated changes in nutritional preference. In contrast, other studies compare GUDs among sites. This is problematic because foragers in these sites may be experiencing unequal risk of predation, therefore confounding the foragers nutritional preferences, and in turn the amount of food left in an experimental GUD tray. Thus, GUD results may not accurately reflect differences in predation risk among those areas. Therefore, accurately measuring risk using the GUD framework may require careful consideration of the experimental design (paired within-sites versus among-sites). Additionally, future studies should consider the nutrient content of foods being used, and, if possible, identify food types and nutrient ratios for which preference does not covary with predation risk.

This study may help explain inconsistent and counterintuitive results reported in many studies, such as lower GUDs measured in high-risk areas compared to low-risk areas. For example, Eccard et al. (2008) found that Myodes glareolus (bank voles) had lower GUDs in risk treatments than controls. Consistent with my hypothesis, they used millet seeds, which have a high concentration of carbohydrates (73%; USDA 2017). Similarly, Yunger et al. (2002) used oats (75% carbohydrates; USDA 2017) to study foraging behavior of Akodon olivaceus (olivaceous field mice), and found that mice had lower GUDs in areas where predators were not excluded than where they were excluded.

My results suggest that carbohydrate-rich foods may decrease the difference in GUDs between high-risk and low-risk sites, and many studies have reported no effect of predator cues on GUDs while using carbohydrate-rich foods (Herman and Valone 2000, Mohr et al. 2003, Orrock and Danielson 2009, Fanson 2010, Gutman et al. 2011, Shapira
et al. 2013, Wasko et al. 2014). Similarly, these results suggest that using a protein-rich food may exaggerate the differences in GUDs between high-risk and low-risk sites, and I was unable to find any example of predator cues having no or a negative effect on GUDs when using a protein-rich food (e.g., sunflower seeds, almonds, peanuts, and mealworms; (USDA 2017).

While most evidence that predation risk can alter nutritional preferences of prey comes from research with grasshoppers (e.g., Hawlena and Schmitz 2010a, Hawlena et al. 2012, Schmitz et al. 2016), there is evidence of this phenomenon occurring in other taxa. Several GUD experiments that used multiple food types that differed in nutrient composition reported that preferences of small mammals for food types changed between high-risk and low-risk microhabitats (e.g., Brown and Alkon 1990, Leaver and Daly 2003, Perrin and Kotler 2005, Abu Baker and Brown 2012, Baker and Brown 2014). Similarly, in a laboratory study, *Rattus norvegicus* (Sprague-Dawley rats) with elevated stress hormones had a strong preference for carbohydrate-rich foods over lipid- or protein-rich foods (Kumar and Leibowitz 1988). Finally, Clinchy et al. (2013) suggest that chronic stress in humans may explain preference for carbohydrate-rich foods when suffering from post-traumatic stress disorder (Carmassi et al. 2015, Klingaman et al. 2016), as well as anxiety and depression (Wurtman and Wurtman 1995, Reeves et al. 2008). Thus, although I tested my hypothesis using spiders and grasshoppers, there is compelling evidence that these results may apply to many other taxonomic groups.

Giving-up density experiments have provided valuable information regarding the non-consumptive effects predators have on their prey, but their outcomes may be confounded by the type of food involved (Bedoya-Perez et al. 2013). My results
demonstrate that predation risk can alter nutritional preferences of prey, thereby
confounding the interpretation of GUD experiments. My results highlight the need to
integrate emerging insights from predation risk and nutritional ecology to maximize the
utility of this classic experimental design in future studies.
### 1.5 Tables and figures

Table 1.1  Results from choice and no-choice experiments

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<td>15.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>type</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results of linear models showing individual and interactive effects of predation risk on consumption. Predation risk was created using a single spider isolated inside of a transparent, vented dish. Consumption was measured for two food types, one that was carbohydrate-rich and the other that was protein-rich. Grasshoppers in choice trials had access to both food types simultaneously, whereas grasshoppers in no-choice trials were presented with only one food type. Results significant below an alpha of 0.05.
Table 1.2 Results summarized from GUD experiment

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Model Effect</th>
<th>d.f.</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giving-up density</td>
<td>Predator density</td>
<td>1,14</td>
<td>1.679</td>
<td>0.216</td>
</tr>
<tr>
<td></td>
<td>Food type</td>
<td>1,14</td>
<td>1.712</td>
<td>0.212</td>
</tr>
<tr>
<td></td>
<td>Predator density*food type</td>
<td>1,14</td>
<td>7.975</td>
<td>0.014</td>
</tr>
<tr>
<td>Giving-up density (P-rich)</td>
<td>Predator density</td>
<td>1,7</td>
<td>5.767</td>
<td>0.047</td>
</tr>
<tr>
<td>Giving-up density (C-rich)</td>
<td>Predator density</td>
<td>1,7</td>
<td>2.210</td>
<td>0.181</td>
</tr>
<tr>
<td>Consumption</td>
<td>Predator density</td>
<td>1,14</td>
<td>1.559</td>
<td>0.232</td>
</tr>
<tr>
<td></td>
<td>Food type</td>
<td>1,14</td>
<td>2.1</td>
<td>0.1693</td>
</tr>
<tr>
<td></td>
<td>Predator density*food type</td>
<td>1,14</td>
<td>9.349</td>
<td>0.009</td>
</tr>
<tr>
<td>Consumption (P-rich)</td>
<td>Predator density</td>
<td>1,7</td>
<td>7.065</td>
<td>0.033</td>
</tr>
<tr>
<td>Consumption (C-rich)</td>
<td>Predator density</td>
<td>1,7</td>
<td>2.677</td>
<td>0.167</td>
</tr>
<tr>
<td>Grasshopper Mortality</td>
<td>Predator density</td>
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<td>5.96</td>
<td>0.029</td>
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<td></td>
<td>Food type</td>
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<td>0.418</td>
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<tr>
<td></td>
<td>Predator density*food type</td>
<td>1,14</td>
<td>2.677</td>
<td>0.124</td>
</tr>
</tbody>
</table>

Results of linear models showing individual and interactive effects of predator density and food type on grasshopper consumption, giving-up density (GUD) and mortality. Grasshoppers were exposed to a gradient of spider density (0, 1, 2, 3, 4, or 5 spiders) while foraging on either carbohydrate-rich or protein-rich foods. Results significant below an alpha of 0.05.
Figure 1.1  Choice and no-choice experiments

Mean consumption (mg/day) of carbohydrate-rich (circles) and protein-rich (triangles) food by individual grasshoppers in the presence and absence of a spider predator. (a) In the choice experiment where both food types were present, grasshoppers ate less protein-rich food in the presence of spiders, while consumption of carbohydrate-rich food was unaffected. (b) In the no-choice experiment where only one food type was present, grasshoppers ate less protein-rich food in the presence of spiders, while consumption of carbohydrate-rich food was unaffected. Error bars represent ± 1 standard error of the mean.
Figure 1.2 Giving-up density experiment

Relationship between predation risk (spider density between 0 and 5) and grasshopper (a) giving-up density (GUD) and (b) consumption using two different food types (carbohydrate-rich and protein-rich). Giving-up density was measured as the amount of food remaining in the food tray at the end of each trial. Consumption was measured as the average food consumed per grasshopper per day. Values for protein-rich food are shown with triangles and values for carbohydrate-rich food are shown with circles. Shading represents 95% confidence intervals.
CHAPTER II
BEHAVIORAL OBSERVATIONS

2.1 Introduction

Predators exert non-consumptive effects on their prey (Hunter and Price 1992, Lima 1998a), including altering their diet choices (Edwards 1983, Rothley et al. 1997, Christianson and Creel 2008, 2010). More specifically, recent work has shown that prey exposed to predation risk increase the amount of carbohydrates they consume compared to prey in risk-free treatments (Hawlena and Schmitz 2010a, b, Schmitz et al. 2016). Several hypotheses have been proposed to explain why predation risk causes prey to switch their diet preferences from protein-rich diets to one that favors carbohydrates. First, predators may force prey into different habitats, such as those that offer refuge due to their structural complexity (Beckerman et al. 1997, Warfe and Barmuta 2004, Christianson and Creel 2008).

In old-field systems, structurally complex plants such as Solidago species generally contain higher proportions of carbohydrates (Hawlena and Schmitz 2010a). Therefore, prey may consume higher proportions of carbohydrate-rich plants consequent to choosing a complex habitat. Second, stress from the risk of being killed and consumed by a predator has been shown to elevate the metabolic rates of grasshoppers in a laboratory (Hawlena and Schmitz 2010a). This causes them to expel more CO₂, and create a carbon deficit in their bodies. Further, elevation and maintenance of this
heightened metabolic rate could explain the carbohydrate preference due to loss of carbon to respiration (Hawlena and Schmitz 2010a). Third, it has been suggested that prey consume more carbohydrates in the presence of predators to maintain a high proportion of carbohydrates in their bodies, as they represent a source of fast burning energy (Hawlena and Schmitz 2010b, Schmitz et al. 2016). Additionally, physiologic stress induces the breakdown of stored nutrients into glucose, allowing stressed animals to have adequate resources to escape predation, and increasing the value of carbohydrates that can replace energy stores (Rovero et al. 2000, Beckman and Muller-Landau 2007, Hawlena and Schmitz 2010a). Therefore, predation risk can cause animals to change locations, elevate metabolic rates, and break down stored nutrients in to fast-burning energy.

All three of these mechanisms offer potential to explain why predator-stressed prey preferentially consume carbohydrates. However, I hypothesize that another mechanism may contribute. Animals that are evading predation may burn more energy than animals at rest (Lima and Dill 1990, Lima 1998b), and coupled with the mechanisms described above, may cause stressed animals to consume foods that can be rapidly converted to energy, such as carbohydrates (Hawlena and Schmitz 2010b). I hypothesize that predation risk causes prey to be more evasive, moving more frequently and covering more distance compared to prey in the absence of risk. This in turn may cause prey to preferentially consume carbohydrate-rich plant tissues that are easily converted into energy (Hawlena and Schmitz 2010b), such as those in the genus *Solidago* (Hawlena and Schmitz 2010a). Additionally, replacing lost calories may become increasingly important in the presence of predators, due to the potential need for rapid
escape. In this way, my hypothesis (H1) is complimentary to current theory (i.e. labile carbohydrates should hold higher value to prey that are stressed by predation due to their rapid assimilation into metabolic energy (Hawlena and Schmitz 2010a)).

To examine grasshopper habitat use, diet choices, and movement patterns, I designed and conducted a behavioral mesocosm experiment that tracked arthropod host-plant use, and coordinates in three-dimensional space. These data allowed me to address the following hypotheses:

H1: Grasshopper movement patterns differ in predator treatments (i.e. move more frequently, cover more total straight-line Euclidean distance)

H2: Grasshoppers in predator treatments eat less frequently compared to predator-free treatments

H3: Grasshoppers in predator treatments eat more Solidago (carbohydrate-rich) plants than grasses (protein-rich)

2.2 Methods

During the summer of 2017, I observed spider and grasshopper behavior in four rectangular mesocosms (60 x 30 x 80 cm) made from wood, and wrapped 80 cm high aluminum insect screen. The bottom of each mesocosm consisted of natural prairie sod that was harvested from a local field site. I drew a 10-cm grid on the front and sides of each of four mesocosms to quantify arthropod location to the nearest cm. Plant communities contained an approximately equal mixture of grass (Schizachyrium scoparium) and goldenrod (Solidago rugosa) species. This mixture was chosen due to S. rugosa being carbohydrate-rich, and grasses generally being nitrogen-rich (Hawlena and Schmitz 2010a). Mesocosms were placed on outdoor table tops exposed to ambient
temperature, light, and humidity for the duration of the experiment. Wild-caught *Tibellus oblongus*, *Peucetia viridans*, and *Melanoplus femurrubrum* were obtained from a field site near Starkville, MS. After transport to the mesocosms, I painted them with fluorescent paint (Testors model paint, Rockford Illinois, USA) to aid in my ability to find them in the canopy (Joern et al. 2006, Barton and Schmitz 2009, Barton 2010). After randomly assigning risk treatments for each block, arthropods were stocked into the mesocosms and allowed to acclimate for at least 24 hours prior to the start of the experiment. Each mesocosm held four 3rd instar *M. femurrubrum* grasshoppers, and two of the four contained an ambush spider predator (*T. oblongus* or *P. viridans*). Therefore, each block had a spider-free and a spider-present treatment.

The x (horizontal), y (vertical), and z (depth) coordinates of each arthropod were recorded to the nearest centimeter at 20-minute intervals between 06:00 and 20:00. Arthropod behavior was recorded at each interval, categorized as eating, climbing, or resting, and the host plant was recorded. Each arthropod was assumed to be performing their behavior (i.e. eating, climbing, or resting) for the entire 20-minute interval (Belovsky and Slade 1986, Barton 2010). At the end of each experiment, arthropods were released. New, wild-caught arthropods were used for every experimental round to maintain independence. The experiment was conducted on 5 separate days, totaling 4 replicates for *T. oblongus*, and 5 replicates for *P. viridans*. Mesocosms without a predator (two trophic levels) serve as a control for testing hypotheses, against which comparison can be drawn between grasshopper movement and diet choice (if feeding) of the predator treatments. All arthropods that died before or during the experimental observations were excluded from analyses.
2.2.1 Movement analysis

To further test the hypothesis that movement patterns differ between control and predation risk groups (H1), I calculated the total distance moved by each grasshopper using a time-step approach (Calenge et al. 2009). To test (H1), I used a Euclidean distance (sometimes called step length (Edelhoff et al. 2016)). This method calculates the straight-line distance between locations (DBL) in 3-dimensional space between each time step, and is suitable for describing animal movement behavior (Franke et al. 2004). The Euclidean distance equation can be seen in equation 2.1.

$$d(p, q) = \sqrt{(p_1 - q_1)^2 + (p_2 - q_2)^2 + (p_3 - q_3)^2}. \tag{2.1}$$

Individual grasshopper distances were averaged within each enclosure on each separate day. Therefore, if an experimental treatment housed 4 grasshoppers, I averaged their total accumulated Euclidean distances before comparing across treatments. This resulted in 19 measurements for grasshoppers (10 control, 9 risk) and 9 measurements for spiders. After obtaining total Euclidean distance measurements, I ran a linear model with total grasshopper Euclidian distance as the response variable and predation risk as a fixed effect. To test for differences in grasshopper response to each spider species, I used a linear model with total grasshopper distance as the response variable and spider species as a random effect. Additionally, I calculated the straight-line Euclidian distance traveled by spiders, and compared means with a Welch’s t-test. Spider presence (i.e., risk) data for both spider predators were pooled for risk analysis due to similar average distances moved (in cm), and therefore were characterized as having the same hunting mode.
(Miller et al. 2014). All analyses were run in the statistical programming language R (R Core Team 2017).

2.2.2 Chi-square analysis

I estimated the treatment effect (spider presence) on observed behaviors and host plants with chi-square goodness of fit tests using the statistical programming language R (R Core Team 2017). Grasshopper behaviors performed on the insect screen (experimental enclosure) were excluded from the diet analysis. I tested the hypothesis that predation risk affected arthropod diet choices by using a Chi-squared test to compare both the overall observed frequency of eating behavior (H2), and the observed frequency of eating behavior on each plant host between control and risk groups (H3). This was accomplished by creating observed frequency tables of grasshopper eating behavior on all hosts, and on each host separately before performing Chi-squared tests. Due to Chi-square analysis being performed on behavioral categorical variables, each grasshopper was treated as an individual. To test for associations between host plant preference and predation risk, a Chi-square test was run on the observed frequencies of all behaviors for each host plant. This was done by creating observed frequency tables for each all behaviors on each plant, and then comparing observed and expected frequencies with a Chi-square test.

I tested the hypothesis that predation risk affected arthropod activity (H1) by using a Chi-square test to compare the frequencies of climbing behavior (on any host, including the cage) between control and risk groups. Additionally, Chi-squared tests were used on the observed frequency of climbing behavior on each plant host between control and risk groups. This was accomplished by creating observed frequency tables of
grasshopper climbing behavior on all hosts, and on each host separately before performing Chi-squared tests.

In addition to climbing analysis, and to provide additional habitat usage metrics, I tested for differences in resting behavior between control and risk groups with a Chi-squared test. A similar method was used to compare resting behavior on each plant host between control and risk groups. This was accomplished by creating observed frequency tables of grasshopper resting behavior on all hosts, and on each host separately prior to performing Chi-squared tests. An adjusted alpha-value of 0.05/12 = 0.0042 was used due to having 12 planned comparisons within the same data frame (e.g. Bonferroni correction). Having multiple comparisons inherently increases the probability of a type 1 error (e.g. falsely rejecting a null hypothesis that is true), and therefore this correction should be applied (Abdi 2010).

2.3 Results

2.3.1 Movement analysis

Individual grasshopper distances were averaged within each enclosure on each separate day. To test for block and cage effects, I ran linear models with total distance moved as the response variable. Block (random-effect) \( F = 1.11, \, d.f. = 1, \, P = 0.30 \) and cage (random-effect) \( F = 0.71, \, d.f. = 1, \, P = 0.40 \) were not significant. The hypothesis that grasshoppers covered more total straight-line Euclidean distance in predator treatments compared to controls (H1) was not supported by DBL analysis \( (F = 0.56, \, d.f. = 1, \, P = 0.46) \) (Fig. 2.1). The effect of spider species on total grasshopper Euclidian distance was also not significant \( (F = 1.31, \, d.f. = 16, \, P = 0.3) \), (Fig. 2.2). Total Euclidian
distance moved by predators was not significantly different for each spider species (Welch’s $t = -0.677$, $d.f. = 4.27$, $P = 0.53$), (Fig. 2.3).

2.3.2 Chi-square analysis

There was no significant difference between observed and expected grasshopper feeding frequency on both plants (H2) ($X^2 = 0.22$, $d.f. = 1$, $P = 0.64$). The same was true for the diet choice analysis (H3): observed frequencies of grasshoppers eating little bluestem ($S. scoparium$) ($X^2 = 1.5$, $d.f. = 1$, $P = 0.22$), and goldenrod ($S. rugosa$) ($X^2 = 0.84$, $d.f. = 1$, $P = 0.36$) did not differ statistically between predation control and risk treatments. There was no significant difference between observed and expected grasshopper feeding frequency on either plant in both predation control and risk treatments (Fig. 2.4).

Observed frequencies of all grasshopper behaviors on both plant hosts did not differ significantly from expected between control and risk treatments ($X^2 = 0.999$, $d.f. = 2$, $P = 0.22$), (Fig. 2.5). The same was true for all grasshopper behaviors on little bluestem, as they also did not differ from expected values ($X^2 = 3.54$, $d.f. = 2$, $P = 0.17$). Observed frequencies of all grasshopper behaviors performed on goldenrod did not differ significantly from expected values between control and risk groups ($X^2 = 7.82$, $d.f. = 2$, $P = 0.02$), (Fig. 2.6).

Observed frequencies of grasshopper climbing behavior on all hosts did not differ from expected ($X^2 = 2.15$, $d.f. = 1$, $P = 0.14$), (Fig. 2.7). This was also true for observed climbing behavior on bluestem ($X^2 = 2$, $d.f. = 1$, $P = 0.16$). There was no significant difference between the observed and expected frequency of grasshopper climbing
behavior on goldenrod plants ($\chi^2 = 2,\ d.f. = 1,\ P = 0.01$), (Fig. 2.8). There were no significant differences between observed and expected grasshopper resting behavior between control and risk groups on either plant host. A complete summary of Chi-squared test results can be found in table 2.1.

2.4 Discussion

Chi-squared analysis revealed that both diet preferences and eating behavior were not statistically different in the presence or absence of a spider predator. Therefore, I failed to reject the null hypothesis that observed behaviors do not differ between control and risk groups (H2 and H3). While other studies have shown treatment effects of predators on diet choices in grasshoppers (Beckerman et al. 1997, Schmitz and Suttle 2001, Barton et al. 2009, Miller et al. 2014), these studies used a different, larger predatory spider (*Pisurina mira*). Although previous studies have found that grasshoppers increase interaction with carbohydrate-rich goldenrod plants while experiencing predation risk (Beckerman et al. 1997, Schmitz and Suttle 2001, Barton et al. 2009), I was unable to detect any significant interactions in the current study.

While my analysis failed to detect any significant interactions, the results leave room for further development of the study system. Multiple possibilities emerge when considering why these results were disparate from other studies using similar systems. The first is that the selected measurement interval is too long (i.e. taking a behavioral measurement every 20-minutes), and this study lacked the resolution needed to detect changes in diet choice patterns across predation risk versus control groups. However, similar designs found significant treatment effects in similar sized areas with the same sampling interval (Barton and Schmitz 2009). Because I was unable to detect any risk
effects, it is also possible that the experimental variable of created risk was confounded by being too high (i.e. risk effects were observed on control and risk grasshoppers), or too low (i.e. actual densities of spiders in the field are higher than in behavioral mesocosms).

Further, the natural occurrence of *T. oblongus* in local prairie ecosystems are very high in the spring (*P. viridans* occurs at similar abundances in summer), at times exceeding 4 per square meter (*personal observation*). Previous studies have used a less common, larger spider (*P. mira*) (Barton et al. 2009, Hawlena and Schmitz 2010a) that occur at lower densities in old fields than *T. oblongus* and *P. viridans* (Brandon Barton, *personal communication*). Although there are no studies that characterize the home range and hunting area of these spider species, anecdotal evidence suggests that a single *P. mira* may be perceived as riskier by *M. femurrubrum* due to their larger size. Since the natural densities of *T. oblongus* and *P. viridans* can reach 4 per square meter in the field where this study took place, multiple spiders should be used in future studies for risk treatments.

I used a similar protocol for observing grasshoppers as other studies, and used a similar number of spiders (Barton et al. 2009, Barton 2010). Because my design and data collection closely match these studies that found significant risk effects, it is unlikely that I measured behavioral metrics in such a way as to confound risk effects. However, it is possible that the metric I chose to quantify arthropod movement (i.e. Euclidian distance) failed to capture the true nature of the predator prey interaction.

The plant community I selected for my study was different than published studies, due to regional variation in prairie community structure. The most important difference in the plant community I used is that the grass species in my study (*S. scoparium*) has a C:N ratio similar to that of *S. rugosa*. This is problematic because emerging theory suggests
that the indirect effects predators have on prey may be mediated through increased
carbohydrate-rich foods (Hawlena and Schmitz 2010b, Schmitz et al. 2016). While this explanation provides valid room for improvement in this study system,
I suspect that my lack of statistical significance is an artifact of low replication.

The lack of statistical power due to low sample size (n = 9), as well as variation
introduced by using two different predator species may have inhibited my ability to
detect significant differences in the current study, if they occurred (Type 2 error). While
similar studies have found significant results with lower sample sizes 6-8 replicates
(Barton 2010), and 5 replicates (Barton and Schmitz 2009), more replication would have
strengthened the analysis, as some studies have reached as high as 30 replicates (Miller et
al. 2014). The natural history of T. oblongus, the spiders become increasingly rare in the
mid-to-late summer (personal observation). For this reason, I used a co-occurring spider,
P. viridans as an additional risk treatment. While the hunting modes may be similar
(ambush), using two spider species undoubtedly introduced extra variation into the study
system.

Although results from the Euclidian distance analysis were not able to detect a
significant risk effect on total distance accumulated by grasshoppers, they were able to
characterize the spider predators used in this study with a similar hunting mode, as total
distances moved by spiders were not significantly different (Fig. 2.7). Other studies have
used a Euclidean distance approach to approximate activity levels in spider predators. For
example, Miller et al. (2014) measured Euclidian distance accumulated by spiders at 3-
minute intervals for one hour. While this study used those data to characterize hunting
modes, it fails to provide Euclidian distance covered in an entire day as does the current
study. However, variance in total accumulated Euclidian distance between spider species was unequal (F-test: $F = 166840$, $d.f. = 8$, $P < 2.2e-16$). Therefore, the hunting mode of the two spiders in this study may be slightly different even though mean distances travelled did not differ significantly (Fig. 2.3).

Although I was not able to detect significant results in the current study, I was able to quantify movement of spiders and grasshoppers over an entire day (dawn – dusk). In addition, I provide recommendations for future studies, including using higher densities of spiders in risk treatments, careful consideration of local plant quality, and estimation of arthropod location to the nearest centimeter in behavioral enclosures. Non-consumptive effects in ecosystems are complicated, however methods developed here are a significant step forward in understanding how predator effects cascade through ecosystems, mediated through herbivore behavior.
## 2.5 Tables and figures

Table 2.1 Results of Chi-square comparisons between grasshopper behaviors and diet choices as a function of predation risk

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Host</th>
<th>Risk</th>
<th>Observed freq.</th>
<th>Expected freq.</th>
<th>$X^2$</th>
<th>d.f.</th>
<th>P-value</th>
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<td>121</td>
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</tr>
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<td>115</td>
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<td>0.07</td>
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<tr>
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<td>Goldenrod</td>
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<td>456</td>
<td>429</td>
<td>3.40</td>
<td>1</td>
<td>0.07</td>
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</table>

Results of chi-square tests showing observed and expected frequencies for behavioral analysis. Arthropods were observed at 20-minute intervals between 6:00-20:00, N = 8. 12 planned comparisons were made, therefore an alpha-value of $0.05/12 = 0.0042$ was used as the threshold of significance.
Figure 2.1  Total grasshopper Euclidian distance by risk

Averaged straight-line Euclidian distances moved by grasshoppers, separated spider presence/absence. Linear model: total grasshopper distance \sim risk. $F = 0.56$, d.f. = 1, $P = 0.46$. Grasshoppers did not move significantly more or less in predator treatments.
Figure 2.2  Total grasshopper Euclidian distance by spider species

Averaged straight-line Euclidian distances moved by grasshoppers, separated by spider species, $F = 1.31$, $d.f. = 16$, $P = 0.3$. Grasshoppers did not move significantly more or less in predator treatments.
There was no mean difference in total Euclidian distance covered between spider species. Welch’s $t = -0.677$, $d.f. = 4.27$, $P = 0.53$. This suggests that hunting modes are similar between these two spider species.
Figure 2.4    Observed and expected frequencies of grasshopper diet choices

Observed and expected frequencies of grasshopper diet choices in both control (risk-free) and predation risk mesocosms. Grasshoppers were able to freely eat both little bluestem (*Schizachyrium scoparium*), and goldenrod (*Solidago rugosa*). There were no differences between groups $X^2 = 1.5418$, $d.f. = 1$, $P = 0.21$. 
Figure 2.5  Frequencies of observed behaviors on both plants, separated by predation risk

Frequencies of observed behaviors on both plants, separated by predation risk. There was no difference observed in the frequencies of overall behaviors. $X^2 = 0.999$, $d.f. = 2$, $P = 0.22$. Presented as for representation of overall grasshopper behavior.
Figure 2.6  Observed and expected frequencies of grasshopper behaviors on goldenrod plants (S. rugosa)

Observed and expected frequencies of grasshopper behaviors on goldenrod plants. Treatment effect (spider presence) did not have a significant effect on grasshopper behavioral interactions with goldenrod plants following Bonferroni correction. $X^2 = 7.82$, d.f. = 2, $P = 0.02$. 
Figure 2.7  Observed and expected frequencies of grasshopper climbing behavior

Observed and expected frequencies of grasshopper climbing behavior in both control (risk-free) and predation risk mesocosms. Grasshoppers could freely climb both little bluestem (*S. scoparium*), and goldenrod (*S. rugosa*) plants. Groups did not significantly differ with respect to predation risk following Bonferroni correction. $\chi^2 = 5.6227$, $d.f. = 1$, $P = 0.02$. 
Figure 2.8  Observed and expected frequencies of grasshopper climbing behavior on only goldenrod (*S. rugosa*).

The observed frequency of grasshoppers performing climbing behavior on *S. rugosa* did not significantly differ between control and risk groups following Bonferroni correction. $X^2 = 6.4286$, $d.f. = 1$, $P = 0.01$. 
REFERENCES


APPENDIX A

ADDITIONAL DATA, ANALYSES, AND INFORMATION
A.1 Artificial diet preparation

The artificial diets in this experiment were based on Lee et al. (2002) and have been used in previous *Melanoplus Femurrubrum* studies (Hawlena & Schmitz 2010; Schmitz et al. 2016). Two different diets were used differing only in their content of protein (P) and digestible carbohydrates (C): a carbohydrate-rich (7% P and 28% C) and a protein-rich diet (28% P and 7% C). The protein content of the diets came from a 3:1:1 ratio of casein, peptone and albumen, and sucrose was the source of digestible carbohydrates. The diets also contained Wesson’s salt (2.5%), cholesterol (0.6%), linoleic acid (0.6%), ascorbic acid (0.3%) and 0.2% of a vitamin mix (Dadd 1961). The vitamin mix included thiamine (1.4%), riboflavin (1.4%), nicotinic acid (5.6%), pyridoxine (1.4%), folic acid (1.4%), myo-inositol (14.1%), calcium pantothenate (2.8%), p-aminobenzoic acid (1.4%), choline (70.4%), and biotin (0.06%) and was prepared prior to making the artificial diets by grinding the ingredients together with a mortar and pestle. Cellulose was used as an indigestible filler for the remaining part of the diet.

I prepared the diets in large glass bowls, using measured amounts of each ingredient. First, the cellulose and casein were mixed together, before adding a solution made from linoleic acid, cholesterol, and chloroform (as a solvent). After the cholesterol was dissolved in the solution, it was added to the cellulose and casein and the ingredients were homogenized using a glass stirring rod. The mixture was left in a fume hood for 24 hours to facilitate evaporation of the solvent, mixing it twice during that time to assist evaporation and eliminate lumps.

The second day, the bowl was removed from the fume hood measured amounts of Wesson’s salt, sucrose, dextrin, peptone, albumen, and ascorbate were added before
blending the diet with a glass stirring rod. The vitamin mix and a solution of 20% ethanol were combined before adding it to the rest of the artificial diet and mixing with a glass stirring rod. The mixture was then spread on large baking sheets, and dried in a 30°C oven for 24 hours. The diets were then homogenized and stored them in a freezer until the start of experiments. The diets were given to arthropods in powder form.

A.2 Growth chamber experiment

I hypothesized that grasshoppers in water-stressed situations would preferentially consume more carbohydrates than proteins. Arthropods have been shown to increase carbohydrate metabolism when they are water-stressed. For example, Marron et al. (2003) found that water-stressed *Drosophila* exhibited lower rates of protein and lipid metabolism, but rates of carbohydrate metabolism were several fold higher. I conducted a choice experiment to test this hypothesis. I used a sample size of 18, but all samples were pseudo-replicated within growth chambers due to it being one experimental unit. Data were analyzed using t-tests. Results may be seen in figures A.1-3.

A.3 Hunting mode survey in recently burned areas

I hypothesized that spiders with different hunting modes would colonize the edges and middle of recently burned areas at different rates. Results presented in figure A.4 show data from the first ten days of sampling after a fire. I had two sites for this study, and each site had 3 pitfall traps on the edges and 3 traps in the middle. In addition to pitfall traps, I sampled the arthropod community using a sweep net and 10 meter transects directly over each pitfall trap location. Results may be seen in figure A.4.
A.4 Giving-up density experiment literature review and meta-analysis

During the summer of 2016, I conducted a formal literature review of published giving-up density experiments. My initial search turned up 246 studies, 135 of which used predator cues (e.g. urine, scat, or sound). I analyzed these 135 studies by calculating the effect magnitude of predator cue addition by using the free software Plot Digitizer. I calculated the effect magnitude of predator addition by taking the natural log of the reported giving-up density with the predator cue, divided by the reported giving-up density without a predator cue. Therefore, with a positive effect magnitude means animals give-up at a higher density of food, and a negative effect magnitude means that animals give-up at a lower density, or ate more of a food source. I then referenced the USDA database for nutrition to calculate the carbohydrate to protein ratio (C:N) of all baits used in the 135 studies of interest (USDA 2017). A linear model with effect magnitude as the response variable and C:N as a fixed effect was not significant: $F = 2.639, d.f. = 132, P = 0.11$. A representation of the linear model may be seen in Fig. A.5.

A.5 Arthropod diversity response to prescribed fire

In October of 2016, I collected arthropods by sweep netting transects across large, approximately one-hectare plots that were subdivided into 6 units. Three units per each plot were burned in June 2016, in an alternate, checkerboard fashion. I sampled arthropod communities in each unit (total 18) with three twenty-meter sweep net transects. Arthropod samples from each unit were combined (3 samples) before pooling data for burned and unburned areas in each large plot. This provided with 3 independent replicates (burned vs. unburned) upon which I drew comparisons of arthropod diversity metrics (species richness). Arthropods were frozen for 3 weeks before they were
identified to family. I analyzed the data by fitting a Michaelis-Menten function \( y = \frac{ax}{b+x} \) to it and constructing rarefaction curves. Rarefaction curves operate on the assumption that for a defined space or area, a given sampling effort will eventually produce no novel species (Gotelli and Colwell 2001). Data were simulated by taking 100 re-samples from the burned and unburned areas in each large plot with replacement, and then fitting the average of results to Michaelis-Menten function. This function returned two parameter value estimates, a and b. The a value represents the asymptote, or the family richness value at which increased sampling effort no longer produces new families. The b value represents the steepness of the curve, where a lower number would represent a more even community (Williams et al. 2007). I was able to generate a smooth curve of Michaelis-Menten mean values that I plotted against sample size (Williams et al. 2007). Therefore, comparing confidence intervals around my parameter estimates allowed me to test my hypotheses that arthropod family richness and evenness differed between burned and unburned areas. I used the statistical programming language R (R Core Team 2017) to re-sample and analyze my data.

Rarefaction analysis revealed higher richness and lower evenness in burned areas for plots A and B (Figs A.6 and A.7). Plot C had the opposite results, where the unburned areas had higher richness and lower evenness at the family level (Fig A.8). Comparisons around parameters with 95% confidence intervals can be seen in Table A.1.
A.6 Tables and Figures

Table A.1 Comparisons of 95% confidence intervals around mean parameter estimates

<table>
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95% confidence intervals around parameter estimates for family richness (a) and evenness (b).
Figure A.1  Consumption of carbohydrates in two humidity treatments

Mg carbohydrates consumed in both humidity treatments. $P<0.001$. Grasshoppers in the high humidity treatment consumed significantly more carbohydrates than grasshoppers in the low humidity treatment.
Proportion carbohydrates consumed in both humidity treatments $p = 0.38$. Grasshoppers did not alter the proportion of carbohydrates consumed due to humidity (relative to protein).
Figure A.3  Surviving grasshoppers (day 5)

Average surviving grasshoppers at the end of the experiment (5 days) p<0.001. The number of surviving grasshoppers in the high humidity treatment was significantly higher than low humidity.
Figure A.4  Hunting mode survey

Results from hunting mode assay using pitfall traps and sweep nets in recently burned areas. Active predators colonize the middle of burned areas before ambush predators.
The effect magnitude of predator cue addition in sampled studies (n = 135) did not have a significant interaction with the C:N ratio of food sources used in those studies. $F = 2.639$, d.f. = 132, $P = 0.11$. 

Figure A.5  Preditor effect magnitude by C:N ratio
Figure A.6  Modeled species accumulation curves for plot A

Species accumulation curve for plot A. Burned areas had a higher (a) value, therefore having a higher estimated richness at the family level. Burned blocks also had a lower evenness estimate (b) meaning that the unburned areas were more even.
Figure A.7 Modeled species accumulation curves for plot B

Species accumulation curve for plot B. Burned areas had a higher (a) value, therefore having a higher estimated richness at the family level. Burned blocks also had a lower evenness estimate (b) meaning that the unburned areas were more even.
Figure A.8  Modeled species accumulation curves for plot C

Species accumulation curve for plot C. Burned areas had a lower (a) value, therefore having a lower estimated richness at the family level. Burned blocks also had a higher evenness estimate (b) meaning that the unburned areas were less even.