

**Trophic Cascades: Trait-Mediated Indirect Interactions Between Wolf Spiders (*Lycosidae*)
and Grasshoppers (*Acrididae: Melanoplus*) in an Old Field Environment**

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Abstract

Trophic cascades are considered important indicators for top-down control of food web dynamics. Although there has been an overwhelming body of evidence supporting the existence of trophic cascades, the mechanism driving this interaction is still debated. Trophic cascades can emerge from the lethal direct effects of exploitation by predators that decrease herbivore abundance, leading to a positive effect on plant biomass. Indirect behavioral effects can also induce a trophic cascade; herbivores will adaptively shift their behavior in the presence of a predator to avoid predation at the risk of starvation. This behavioral shift in foraging time can also result in a positive effect by the predator on plant biomass. I conducted a field and laboratory experiment in an old-field ecosystem composed of herbaceous vegetation, grasshopper herbivores, and predatory spiders to evaluate which mechanism drives trophic cascades. I used four treatments to compare the effects of multiple trophic-level interactions on plant biomass. A control treatment of vegetation only, a two trophic-level interaction (grasshoppers and vegetation), and two different three trophic level interactions: the presence of both predatory spiders to examine density-mediated effects, and risk spiders with their chelicerae glued to examine trait-mediated effects. A lab experiment was conducted to examine grasshopper behavior in the presence of both a predator and risk spider, and if food quality had an effect on this interaction. Both risk and predator spiders decreased the impact of grasshoppers on plant biomass, and reduced grasshopper survivorship indicating evidence of a trait-mediated trophic cascade. At the behavioral level, grasshoppers exhibited anti-predator behavior at the expense of reduced food intake and possible starvation risk. Food quality had no effect on the survival of grasshoppers because in both treatments the predator was being avoided at the sacrifice of foraging. My results indicate that the trait-mediated indirect effects induced by a predator reduced grasshopper herbivory, resulting in a trophic cascade.

Introduction

Grasshoppers (*Orthoptera: Acrididae*) are an important component of grassland ecosystems, altering nutrient cycling processes in some instances, and are an important food source for grassland birds (Belovsky 2000, McEwen and DeWeese 1987). Despite their positive role in the ecosystem, they have many negative effects as well. Grasshoppers can outbreak in large numbers that can cause widespread economic damage and reduce plant productivity (Branson *et al.* 2006). Therefore, understanding grasshopper population dynamics is crucial for the successful management of pest grasshopper species.

Since grasshoppers can reduce plant biomass and productivity, research has often focused on food web dynamics. Food web dynamics are an important ecological focus that help ecologists better understand the natural environment and how species interact. Food web dynamics show how energy flows from one trophic level to the next via exploitation and provide a means to think about community organization. Trophic interactions can indirectly affect plant biomass through the regulation of herbivore populations (Laws & Joern 2012). Predators can reduce prey abundance through density-mediated factors, or indirectly through trait-mediated factors such as "fear", affecting time budgets and foraging activity leading to reduced time foraging and reduced plant tissue eaten (Danner & Joern 2003; Schmitz *et al.* 1997). Consequently, plant biomass may increase as predators reduce prey densities or foraging time. For instance, both direct and indirect effects of spider predators on grasshoppers can lead to trophic cascades in grassland ecosystems (Schmitz *et al.* 1997; Schmitz 1998).

The lethal direct effects of predators on prey in a grassland ecosystem cascade through trophic levels, where one species influences another indirectly through a third. This type of interaction is referred to as a density-mediated indirect effect, and a trophic cascade is an example of this food web interaction (Schmitz *et al.* 2004). An abundance of predators such as spiders may reduce the population of herbivorous grasshoppers, which in turn will lead to a positive effect on grasses because there are fewer grasshoppers to forage on them. This could lead to an overall greater abundance of plant biomass in grassland ecosystems indirectly through the predator. Another type of trophic cascade may arise from nonlethal effects of the predators presence which can cause an adaptive shift in prey behavior. These changes in prey behavior, known as risk effects or a trait-mediated indirect interaction, are the result of the prey altering foraging time as a trade-off to avoid predation. However, this behavioral shift may also increase

the likelihood of the grasshopper starving; a detrimental trade off. (Belovsky *et al.* 2011, McNamara and Houston 1987). The trade-off induced by the predator can involve a habitat shift to poor-quality resources for shelter at the expense of high-energy intake in highly risky habitats (Schmitz *et al.* 2004). Risk effects can be examined by disabling the predators mouthparts with beeswax so it can induce fear, but not lethally subdue prey (Schmitz *et al.* 1997). Spiders with their chelicerae glued together are commonly referred to as risk spiders, and ones without altered mouthparts are referred to as predator spiders. Both density- mediated and trait- mediated effects can have a strong influence on how grasshoppers forage. This top-down control by common predators like wolf spiders is shown to alter plant biomass by the regulation of foraging on grasshoppers (Laws and Joern 2012). Plant community dynamics can also be altered by the presence of a predator in the predator-herbivore-plant trophic cascade model. To avoid the risk of mortality, grasshoppers will abstain from eating grass and seek refuge in forb species, causing high amounts of damage to them and reducing their abundance relative to grass species.(Schmitz 2003, Beckerman *et al.* 1997). This is only the case for some species however.

Food quality can also effect survivorship of grasshoppers (Jonas & Joern 2013; Joern & Behmer 1997). High quality plants are considered ones that have a low C:N ratio (high nitrogen compared to low carbon)(Strengborn *et al.* 2008). If high quality food is available in the presence of spiders, grasshoppers may still be able to obtain sufficient nutrients even if less food is eaten, and thus survive. More nutrients can be consumed in a shorter amount of time, limiting time exposure to predation (Danner & Joern 2004). If low quality food is present, survivorship decreases (Oedekoven and Joern 2000).

The purpose of this study is to examine if wolf spiders (*Lycosidae*) can induce a trait-mediated or density-mediated trophic cascade on several species of *Melanoplus* grasshoppers,

and also if food quality can affect prey foraging and survivorship. Specifically, the amount of food eaten by grasshoppers (high quality or low) in the presence or absence of a spider. Several hypotheses were tested in both a field and laboratory experiment. (1) In field enclosed grasshopper populations in the presence of a predator and risk spider, grasshopper foraging will decrease, resulting in greater plant biomass. (2) In the absence of spiders, field enclosed grasshopper survival will increase and plant biomass will decrease. (3) In a lab experiment, grasshoppers will forage less when in the presence of a spider with high quality and low quality food availability, and more high quality food will be eaten to avoid starvation. (4) Grasshoppers in contact with risk and predator spiders will respond by changing their position in the cage above the floor to escape predation, sacrificing feeding.

Methods

A field and lab experiment was conducted at the University of Notre Dame Environmental Research Center (46° 13' N, 89° 32' W, Gogebic County, MI, and Vilas County, WI) over July 2014. Several old-field environments persist in this region dominated by northern hardwood forests, which serve as a habitat for northern grasshopper species. Northern spur-throat grasshoppers (*Melanoplus borealis*) and Dawson's grasshoppers (*Melanoplus dawsoni*) were selected as study species because they are closely related and were readily abundant when collected by sweep-netting. *M. borealis* were used in the field experiment, and *M. dawsoni* were used in the feeding trials. This was done because the experiments started at different times, and the earlier emerging species (*M. borealis*) was the only one available at the time. Both species are univoltine, generalist feeders, meaning they feed on both grasses and forbs (Pfadt 1994). *M. borealis* is a pest species that can reach high densities in parts of its range. Early (I-III) instar stage nymphs were used due to their small size; wolf spiders generally cannot subdue larger

developmental stages. Northern wolf spiders (*Alopecosa aculeata*) and forest wolf spiders (*Hogna frondicola*) were used as predators because they were the only species to fall into the pitfall traps used to collect the spiders.

Field Experiment

A field experiment was conducted in an old-field environment aptly named "grasshopper nation". Twenty field enclosures (0.35m²) were set up in grasshopper nation and assigned one of four treatments by using a random numbers table, and replicated five times: (1) enclosures with only vegetation to serve as a control, (2) grasshoppers and vegetation, (3) vegetation, grasshoppers and spiders, and (4) vegetation, grasshoppers, and spiders with their chelicerae glued with beeswax to prevent them from subduing prey and only acting as intimidation. This experimental design allows me to determine whether the effects of spiders on grasshoppers are density- or trait-mediated. The enclosures were stocked with 10 grasshoppers and 1 spider depending on the treatment. Counts of how many live grasshoppers remained were taken every 2-4 days to quantify survivorship. Vegetation was clipped at ground level after 3 weeks when the experiment was terminated due to time constraint. The vegetation was sorted into grasses and forbs, dried at 61° C for 24 hours, and weighed.

Feeding trials

The lab experiment consisted of 3 different treatments of grasshoppers and spiders: (1) grasshoppers as a control, (2) grasshoppers and spiders, and (3) grasshoppers and spiders with their chelicerae glued, again to prevent lethal interactions. All experiments were performed with two different qualities of food, and replicated four times. A high quality food source was acquired from a 3×3m plot of grass fertilized at 6g of nitrogen per m², and a low quality food

source from the same size plot of short grass growing in rocky soil with an abundance of lichen surrounding it. Grass was clipped at ground level, dried and then weighed. Six ten-gallon aquariums were used as cages, and each was stocked at a density of ten grasshoppers to one spider. Grasshoppers were starved twelve hours prior to tests, and were then presented with one gram of either high or low quality grass. The trials lasted six hours, and during a one hour period, measurements of position in the cage, and if the grasshopper was foraging were taken. After the trials, the food was weighed again to determine the amount eaten in the presence or absence of a risk spider or a lethal predator spider.

Statistical Analysis

Survival data were analyzed for day ten- July 3rd and the final day of the field experiment when vegetation was clipped, day twenty-three- July 16th respectively. A one-way ANOVA was used to analyze survivorship followed by Tukey's post-hoc analysis to examine if a significant difference was detected between treatments ($\alpha=0.10$). Proportion survival measures were arcsine transformed to normalize values. The tenth day analysis was included because in all cages with grasshoppers, the populations began to level off and reach an asymptote (Figure 1). Biomass was analyzed using a one-way ANOVA followed by Tukey's post-hoc analysis for both grasses and forbs ($\alpha=0.05$).

The amount of grass eaten in feeding trials was analyzed using 3 separate independent t-tests: low quality vs. high quality food eaten was compared in each three treatments: (grasshoppers only, predator spider, and risk spider). Also, the average amount of grass eaten after the trials ended was analyzed using a one-way ANOVA followed by a Tukey's post-hoc analysis across the three treatments without taking food quality into account to determine if just

the presence of a spider affected the amount eaten. A Pearson's chi-square test was performed on the position/behavior data recorded in the trials. Behavior in the presence of low quality and high quality food was quantified. The statistical programs R, and SYSTAT 13 were used for all statistical analyses.

Results

Field Experiment

There was no effect of spiders on the final proportion of surviving grasshoppers ($F_{2,12}=0.068$, $p=0.9344$). However, predator spiders did reduce grasshopper survival by 33% halfway through the experiment at day ten ($F_{2,12}=2.831$, $p=0.0984$) (Figure 1.). Biomass analysis showed that there was a significant difference in the weight of grass remaining after the experiment across treatments ($F_{3,16}= 14.26$, $p<0.001$) (Figure 2). Tukey's post-hoc analysis showed that the differences in grass biomass were between grasshoppers ($p<0.001$), predator ($p=0.005$), and risk spiders ($p=0.004$) compared to the control. The difference in biomass between predator spiders and grasshoppers was marginally significant ($p=0.13$). There was no difference in the weight of forbs remaining after the experiment across treatments ($F_{3,16}=1.574$, $p=0.235$).

Feeding Trials

There was no difference in the amount of food eaten by grasshoppers only ($t = 0.8593$, $p=0.4232$), in the presence of a predator ($t=0.2673$, $p=0.6002$) and risk ($t=1.7369$, $p=0.9304$) spider when offered the two different food qualities. Meaning, food quality had no effect on the amount of food eaten. However, when summed across food treatments and when food quality was not taken into account, there was more grass remaining in the spider treatments than grasshopper

only ($F_{2,21}=10.18$, $p<0.001$) (Figure 3). Tukey's post-hoc analysis showed that there was a difference in the amount eaten between grasshoppers in the presence of risk spiders ($p=0.0021$), and grasshoppers in the presence of a predator spider ($p=0.0024$). Behavioral data showed that there was a significant difference in position in the cage when a spider was present in both low ($\chi^2 = 27.8692$, $p<0.001$) and high quality foods ($\chi^2 = 31.5067$, $p<0.001$) (Figures 4&5).

Discussion

The results of the field experiment are consistent with my hypothesis that the presence of a spider would decrease grasshopper foraging, thus increasing plant biomass. There was a 35% increase in grass biomass of enclosures treated with spiders compared to grasshopper only enclosures. This shows evidence of a trophic cascade in the spider-grasshopper-grass food web pathway. The presence of a spider caused a behavioral shift in grasshopper foraging to avoid predation risk, suggesting a trait-mediated trophic cascade. Schmitz *et al.* (1997) found similar results to my experiment, that there was a significant decrease in grass biomass in the two-level trophic system, and an increase in grass biomass in the three-level trophic system. Forb biomass showed no significant difference between treatments in their experiment as well, which could be because plant-plant competitive interactions are not linear; the abundance of forbs would not be expected to compensate for a lack of grass and vice-versa.

Since survivorship did not vary greatly between treatments, there is no evidence of a density-mediated trophic cascade. Survivorship most likely leveled off towards the end of the experiment due to the fact that when the last few counts were taken, all individuals grew into adults that are too large for a wolf spider to subdue. The factor that contributed most to the increase in plant biomass in the spider enclosures is the reduced foraging induced by fear from

the presence of the spider. (Schmitz 1998). Both the predator and risk spider enclosures had similar amounts of grass remaining after the experiment (Figure 2). Schmitz *et al.* (1997) had also found that the effects of risk and predator spiders on trophic dynamics were similar. Beckerman *et al.* (1997) found that grasshopper behavior was the same in the presence of a risk and predator spider. This suggests that the predator spider that was able to lethally subdue prey had about the same effect on biomass as one that could not. The predator spider over time was most likely unable to subdue more grasshoppers because their size kept increasing, but it seems that the grasshoppers still behaved in a fearful manner. Without a predator to reverse the effects of a herbivore, grasshoppers could forage freely which led to the overall lower net biomass in the two-level trophic system.

It should be noted however that progressively throughout the data collection period, a substantial number of *M. borealis* were infected with two different ectoparasitic mites. *Eutrombidium locustarum* are red mites that commonly parasitize grasshoppers and attach to the wings and body, feeding on their hemolymph. Another type of mite which was much more abundant on *M. borealis* is not formally named, and attaches to the appendages of grasshoppers (Belovsky *et al.* 1998). When the last count was taken at the end of the experiment, all individuals were infected. The severity of infection ranged from mild to severe, some individuals had over 60 mites on the appendages alone. The mites were not present when the grasshoppers were collected and used in stocking the cages, however their small size and abundance at grasshopper nation could have led to them infiltrating the cage. Despite their abundance, their presence has been shown to have little effect on resource consumption (Anderson - unpublished data). However, they can affect survivorship, which may explain why there was no significant

difference observed on survivorship in the field enclosures between the treatments, all individuals were infected. (Branson 2003).

The results of the feeding trials done in the laboratory are consistent with my hypothesis that there would be significantly less food eaten when in the presence of a spider, and that there would be an adaptive shift in predator avoidance behavior seen as position in the cage. In the presence of a spider, grasshoppers avoid the base of the aquarium where the food was placed. The results of the food quality test do not support my hypothesis that there would be more high quality food eaten to avoid starvation. There was no difference in the amount of food eaten between food qualities across all three treatments: grasshoppers only (two trophic-level), grasshoppers and predator spiders (three trophic-level), and grasshoppers and risk spiders (three trophic-level). However, significantly less food was eaten in the three trophic level treatments with both high quality and low quality foods as a resource compared to the two trophic -level treatment. This can be linked to the behavioral data that shows a difference in position in the cage when there was a spider present. There were more grasshoppers on the sides, ceiling, and structure in the cage when a spider was present than absent. In the two trophic-level system treatments, there was an 80% increase in the amount of grasshoppers on the base of the cage compared to the three trophic-level system. There were also less grasshoppers foraging in the cages with spiders, which correlates with the data on the amount of food eaten across the treatments. Since the majority of individuals were congregated away from the base of the cage where the food was presented, there was less food eaten in spider cages than cages with only grasshoppers. The two-trophic level system did not have the predator to influence foraging, and therefore more food was eaten.

In this study, I experimentally evaluated the probability of two mechanisms that have been shown to induce trophic cascades in grassland ecosystems. First, spiders can indirectly benefit plants through their direct lethal effects on grasshoppers. Second, spiders can indirectly benefit plants through an adaptive shift in predator avoidance behavior by prey leading to reduced foraging time. The results of my study show that the second mechanism (a trait-mediated indirect interaction) is the driving force in a trophic cascade. This conclusion is based on measurements of grasshopper behavior, foraging in the presence of a spider, and biomass across three trophic levels: single (vegetation only), two-level (grasshoppers and vegetation), and three-level (spiders, grasshoppers, and vegetation). This study suggests that indirect effects at the behavioral level among herbivore populations that comprise a majority of the grassland food web, are possibly responsible for community level indirect interactions like a trophic cascade. The implications of this study can be related to larger food chains that have mainly been examined for density-mediated interactions, and for biological control of outbreak grasshopper species. If trait-mediated effects can happen in a small-scale food chain, larger food chains may have stronger interactions. More complex food webs could also be more reticulated and thus the changes in interaction strength could be highly variable. The regulation of herbivory in this old-field food web shows the need to conserve natural enemies in natural rangeland systems. Future studies could possibly examine the effects over a longer time period since during the study there was a time constraint, and perform studies in a more controlled environment to eliminate the prevalence of ectoparasites.

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Figures

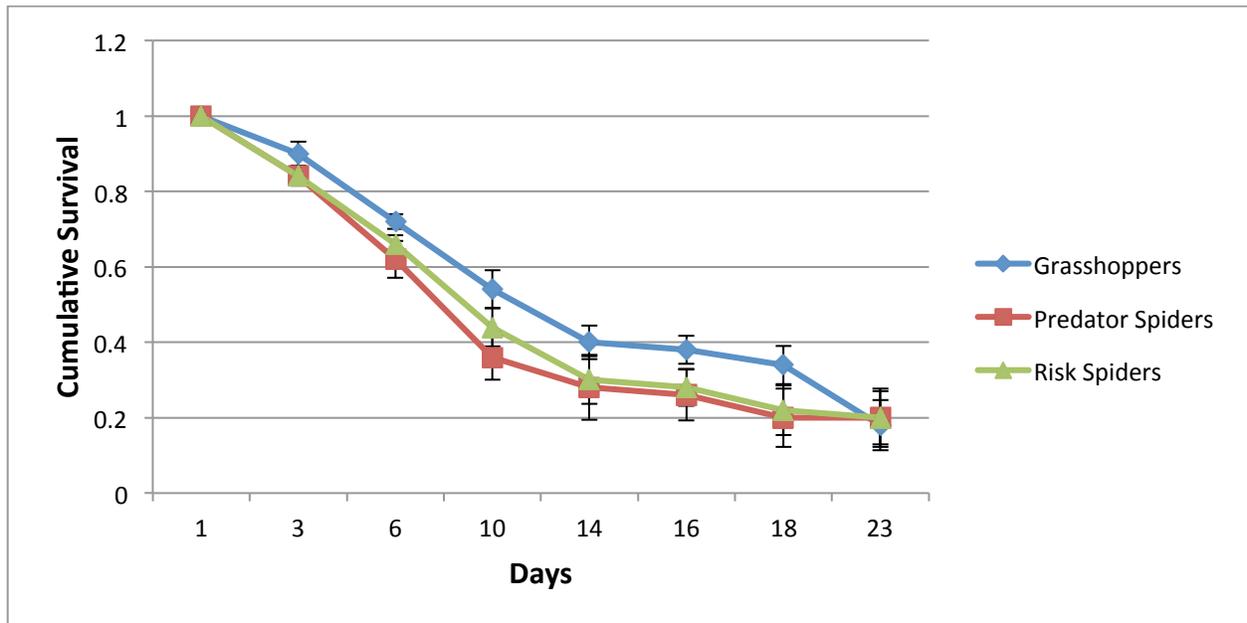


Figure 1. Cumulative survival of grasshoppers in field enclosures over time (proportion out of ten \pm 1 SE). There were a lower proportion of surviving grasshoppers in cages treated with spiders at day ten. The final proportion surviving did not differ among treatments.

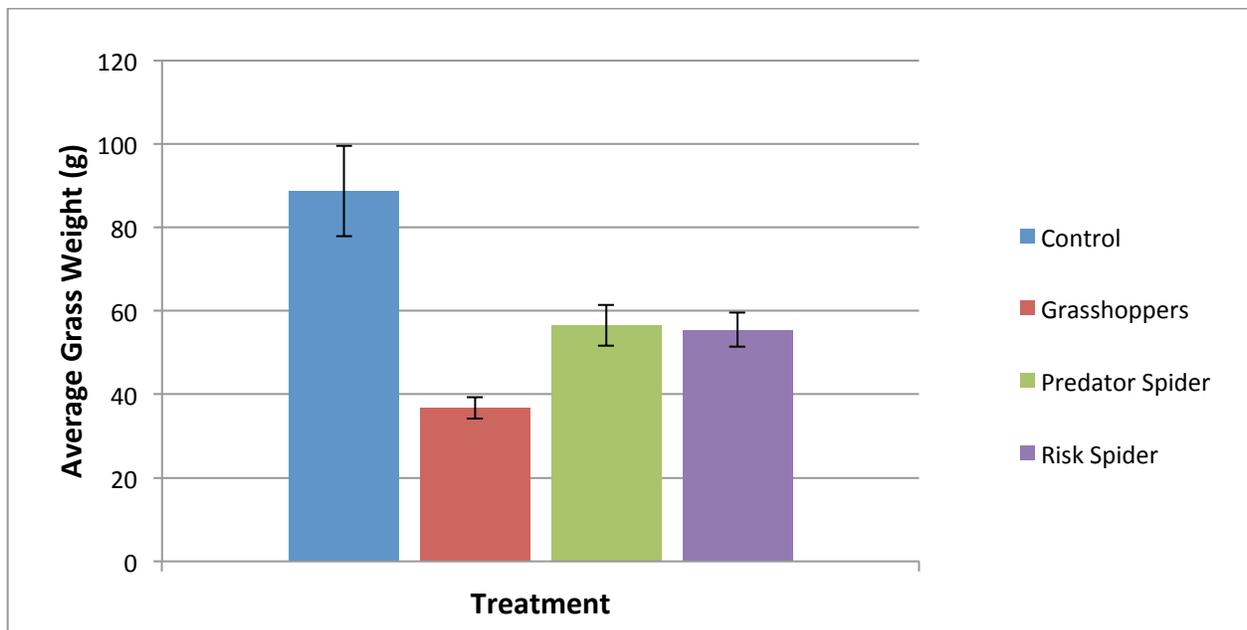


Figure 2. Average grass biomass across treatments after the experiment was terminated (\pm 1 SE). Grasshoppers reduced grass biomass, but grasshopper herbivory was reduced in the presence of a predatory and risk spider.

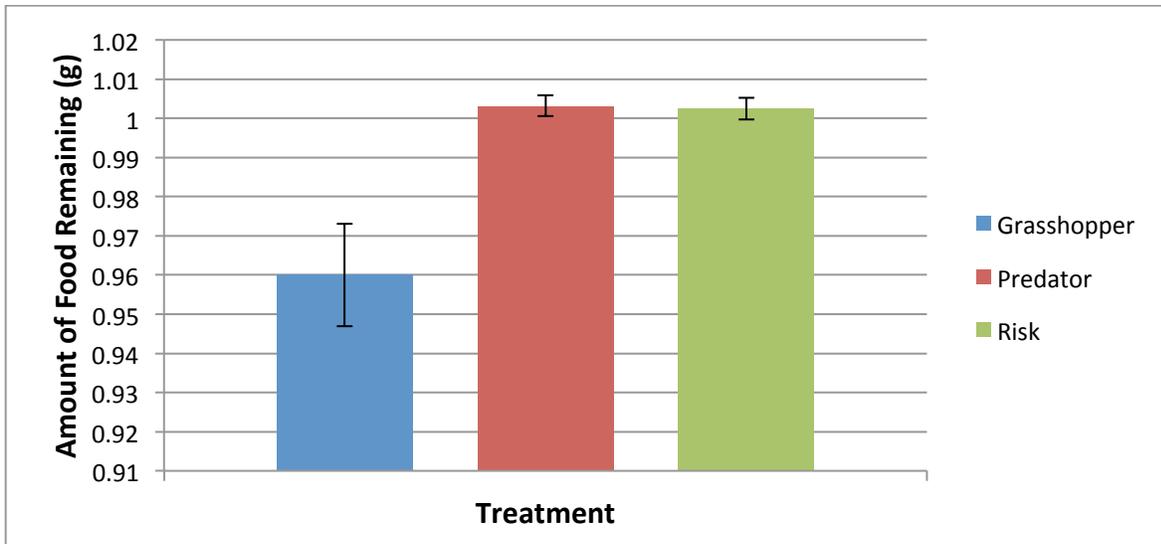


Figure 3. The average amount of food remaining in grams after food trials when food quality was not taken into account (± 1 SE). Grasshoppers foraged less when in the presence of a spider, thus there was more food remaining in these treatments.

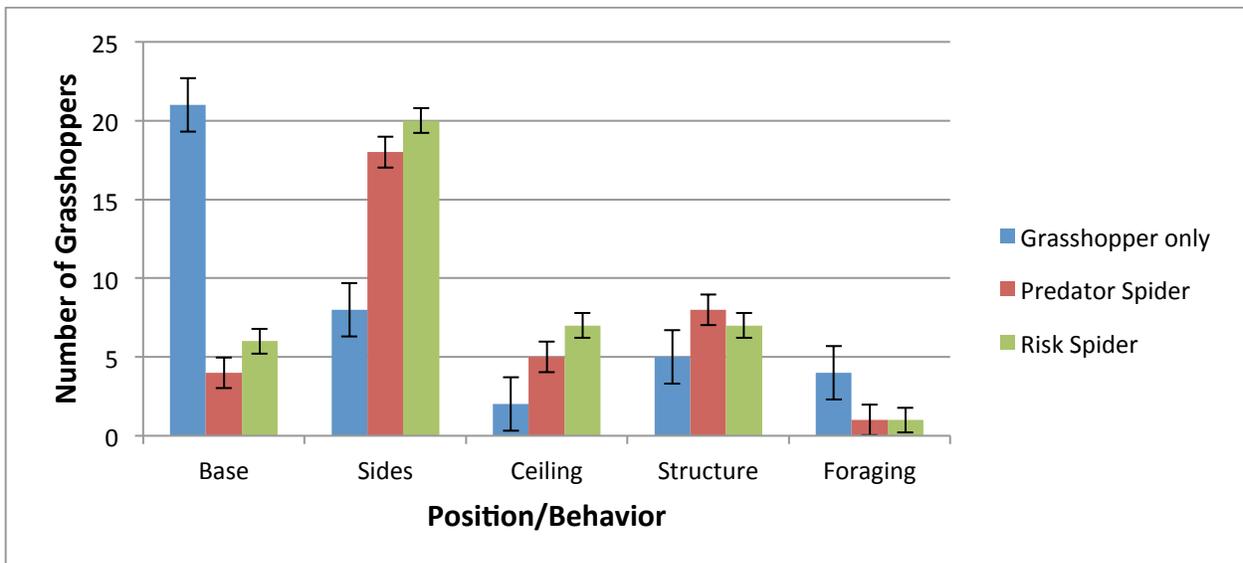


Figure 4. Position and behavior of grasshoppers when fed low quality food across three treatments (± 1 SE). There were a significant amount of grasshoppers congregated around the base of the aquarium, and foraging in the grasshopper only treatments compared to the amount on the sides, ceiling, and structure in the spider treatments.

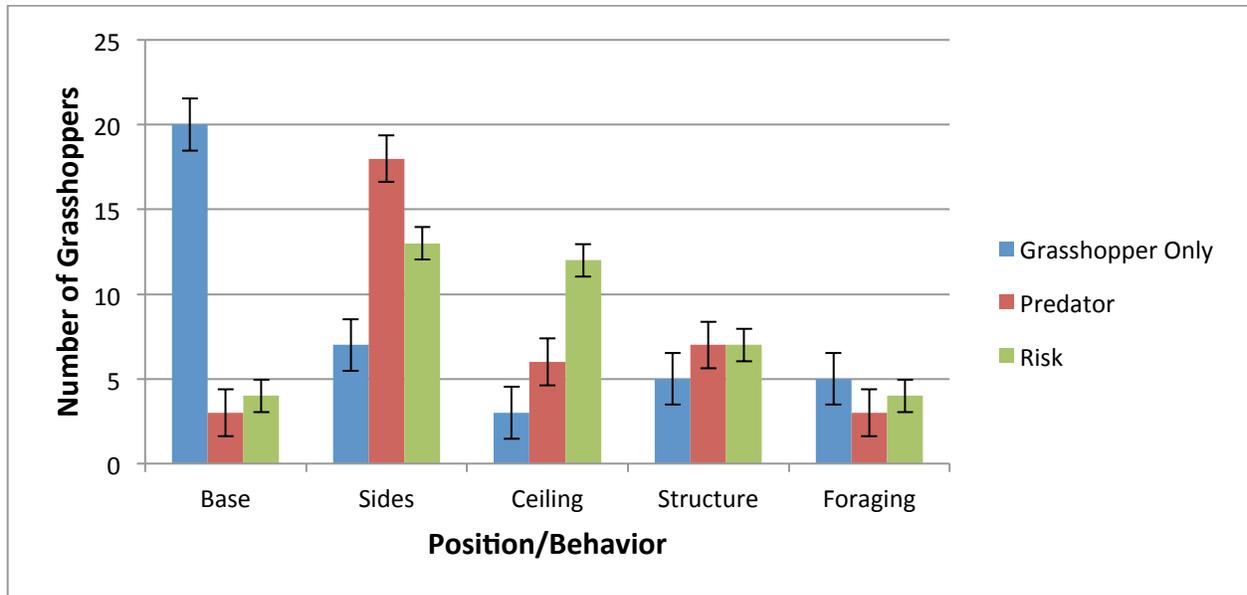


Figure 5. Position and behavior of grasshoppers when fed high quality food across three treatments (± 1 SE). There were a significant amount of grasshoppers congregated around the base of the aquarium, and foraging in the grasshopper only treatments compared to the amount on the sides, ceiling, and structure in the spider treatments.