

The influence of avian predator scent cues on the presence and density of grasshoppers in  
grassland habitats in western Montana

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**ABSTRACT**

Predation risk can influence the distribution and behavior of potential prey. Predation by vertebrates is known to influence the behavior of grasshoppers, and predator scent cues, such as urine and feces, has been known to deter some vertebrate prey species. This research assessed whether grasshoppers have an avoidance response to the presence of chemical cues, droppings, of avian predators. The objective was to see if bird droppings has a negative effect on the density of grasshoppers in an area of grassland. Paired field comparisons between areas with and without bird droppings were done on the National Bison Range and Ninepipe National Wildlife Refuge in western Montana. The hypothesis that the density of grasshoppers in an area with bird droppings present will be less than that of an area without the droppings present was supported. Grasshoppers were found in fewer numbers in areas where droppings were placed compared to similar areas without them present, indicating that grasshoppers were able to detect and respond to the droppings by avoiding the area.

Grasshoppers are some of the most important pests to the economy. Grasshoppers are also an important component of grassland ecosystems across much of the United States, playing a role in nutrient cycling and serving as a critical food supply for wildlife (USDA, 2016; Belovsky and Slade, 2000). Grasshoppers may speed up nitrogen cycling and increase total plant abundance by changing the amount and decomposition rate of plant litter, but under some conditions, grasshoppers may decrease nutrient cycling and plant abundance (Belovsky and Slade, 2000), which may have implications for grazers and those dependant on grasses or grasslands. Grasshoppers can and are known to have reached outbreak densities in the Western North American grasslands, which has led to significant negative impacts on the grazing industry (USDA, 2016). Grasshoppers also have been found to sometimes reduce the foraging rate of livestock as well as wildlife (Belovsky, 2000), which can lead to many communities wanting to deter or eradicate them from certain areas.

There are many groups that want to deter grasshoppers, and insecticides are often the method of choice (Royer, 2004). These pesticides can be very effective in eradicating grasshoppers from an area, but totally removing grasshoppers has negative effects on the wildlife and ecosystem that depends on them since grasshoppers can be very beneficial to an ecosystem (Ryan, 2017). This suggests a need for an alternative way of removing or deterring grasshoppers that does not completely eradicate them.

For some vertebrate herbivores, the use of predator urine or feces has been used to deter them from specific areas or influence their feeding behavior to effectively decrease their damage to vegetation (Nolte, *et al.* 1994; Swihart, 1991). A study compared the repellent capabilities of predator urine and artificial repellants in a variety of herbivores and found that predator urine

was an effective repellent of the vertebrate herbivores (Nolte, *et al.* 1994). Another study found that the presence of urine from bobcats and coyotes, both being predators, reduced the browsing of Japanese yews compared to the rabbit and human urine, neither being predators to the yew, which did not reduce browsing (Swihart, 1991).

Predator scent and chemical cues has shown to be an effective deterrent for larger herbivores, but is it possible to apply it to insect herbivores? Grasshopper are known to exhibit behavior changes when faced with the threat of predation which can have a negative effect on their herbivory (Belovsky, *et al.* 2011; Schmitz, *et al.* 1997). Grasshoppers were able to sense the presence of predators, such as birds and spiders, and were found to change their behaviors to avoid predation. Additionally, naïve cricket nymphs have been found to avoid chemo tactile cues of predacious spiders when the spiders were previously fed with crickets (Kortet & Hedrick, 2004). This suggests that chemical cues found in predators' waste materials can be sensed by crickets and leads to behavioral changes. Since grasshoppers are capable of olfactory reception and communication as well as other chemoreception (Whitman, 1990), similar avoidance behaviors may be possible to observe when presented with predator wastes.

This research addresses this question: can predator excretions be used to reduce grasshopper herbivory and repel them from an area? The objective was to see if bird droppings has a negative effect on the density of grasshoppers in an area since birds are a major predator they are know to avoid, and bird waste is easily accessible. The hypothesis was that with the presence of bird droppings, a predator scent cue, the density of grasshoppers in an area will be less than that of an area without the cue present. The results from this research may have the

capability to lead to further research into finding less harmful ways for people to deter and reduce the herbivory of grasshoppers.

## **MATERIALS AND METHOD**

To compare the response grasshoppers had to areas with bird droppings present versus areas with an absence of bird droppings, pairs of 0.3632 m<sup>2</sup> rings were placed 5 meters apart in areas of seemingly similar grassland vegetation for data collection. Vegetation composition data of each ring was taken and compared to ensure ring pairs contained similar habitat. Data was collected from 20 pairs of rings at 16 different sites on the National Bison Range and Ninepipe National Wildlife Refuge in western Montana (Figure 1). Sites were selected based on their variation in grassland vegetation between each other and the presence of grasshoppers. Pairs of rings were placed somewhat randomly at each site using an 8 sided die, each side representing a cardinal or ordinal direction, to throw the initial ring away from site center. The second ring was placed about 5m away in a similarly vegetated location.

Once the rings were placed, they were acclimated for 15 minutes. After acclimation, grasshopper density was assessed in these control rings using the pointer method where the handle of a sweep net was used to disturb the grass inside the ring to make the grasshoppers hop out of the ring and be tallied. Immediately after this, a sweep net was then swept over and around each ring 10 times to collect the grasshoppers in and around each ring. The collected grasshoppers were identified to species, counted, and released from where they were collected. This data was intended to be used to calculate diversity metrics for each ring. This estimates the natural grasshopper population densities and diversity at each site and acted as control group data.

After the initial grasshopper population data was collected, each ring in a pair had a wetted cotton ball placed in the center. The first ring in a pair had at its center a cotton ball that was half wetter with a bird droppings and water solution. Bird droppings were collected for this by means of scraping dried droppings into a jar, and were collected near the sites to ensure they came from birds species found around each site. The bird poop solution consisted of about 1g of dried scrapings for every 50ml of water and was stored in a jar where cotton balls were dipped half way. The second ring in a pair was treated similarly, but with a cotton ball that was half wetted with just water by means of dipping the cotton ball into a jar of water and immediately placing it in the center of the ring. This water treatment allows this experiment to account for any effect the presence of a cotton ball or water may have on the grasshoppers, allowing for clearer analysis of the grasshopper response to the bird droppings. Once the cotton balls were placed into the rings, they were left to acclimate for 15 minutes. After the acclimation time, the same pointer method and sweep net method were used to assess grasshopper density and to identify/count species respectively for each ring in a pair, like was done after the first acclimation with the empty control rings with no cotton balls.

Analysis of the data was done using RStudio. A paired t-test was used to compare the grasshopper densities in each empty ring in a pair to ensure that the comparison between the rings was between adequately similar locations. The empty ring grasshopper densities were then averaged for each site to be used as the control metric in analysis. The diversity metrics from the sweep net data were not calculated nor statistically analyzed due to lack of sufficient data collected, so the analysis became primarily focused on grasshopper density. Normality of the grasshopper density data for each ring treatment, control, water, and bird droppings, was

assessed. A one-way ANOVA was conducted with the grasshopper densities of the three ring treatments. A Tukey's honestly significant difference (HSD) post-hoc test was conducted after the ANOVA.

## RESULTS

Normality was found in the grasshopper densities for each ring treatment, control, water, and bird droppings (p-value = 0.4507, p-value = 0.168, p-value = 0.1383). The paired t-test revealed no significant difference in grasshopper density between the two empty hoops in the pairs at each site (Figure 2; p-value= 0.8336). The One-way ANOVA showed that the effects of the treatments on grasshopper density was significant ( $F(2,57) = 8.077$ ,  $p = 0.000816$ ). The Tukey's HSD test revealed that the average grasshopper density of the ring treated with bird droppings ( $M = 3.716960352$ ) was significantly different from that of the water treated rings ( $M = 7.296255507$ ; adjusted p-value = 0.0311498) and the empty control rings ( $M = 9.154735683$ ; adjusted p-value = 0.0006215). The empty control rings did not significantly differ from the rings with water treated cotton balls in terms of their average grasshopper densities (Figure 3; adjusted p-value = 0.3732084).

## DISCUSSION

The results of this analysis support the hypothesis. In the empty rings of each pair, the lack of significant difference between grasshopper densities, as well as the observed similar vegetation composition, has ensured a fair comparison and analysis between rings at each site. Of the average grasshopper densities found between the different ring treatments, control, water, and bird droppings, it was the bird droppings treatment that was found to be significantly different from the other two, supporting the hypothesis. When assessing the mean grasshopper

densities of each treatment, the rings containing cotton balls contaminated with the bird droppings solution had on average significantly fewer grasshoppers present than the empty rings or wet cotton ball rings (Figure 3). Since the water treated rings did not differ significantly from the empty control ring, the presence of the cotton ball and water did not seem to interfere with the analysis of the effects of the bird droppings.

Additionally, the order in which data was collected may raise questions about its effect on the results, since the empty control data was taken before the data for the two treatments, thus disturbing the area in a way that could have potentially interfered with the results. However, the acclimation time proves to be sufficiently adequate in compensating for this as seen by the lack of significant difference between the control rings grasshopper densities and the water treated rings densities. This indicates that not only did the presence of the cotton ball and water not significantly affect the results, but neither did the order in which data was collected. It can be assumed that the presence of the bird poop can be associated with the observed decreased density of grasshoppers within the rings, and these results were not significantly interfered with by the presence of the cotton ball and water, nor the order in which data was collected.

The results of this study indicate that grasshoppers tend to avoid areas with bird droppings present more than areas where they are absent. Since the droppings were from predatory birds, and they can be considered a cue to the nearby presence of these predators, the grasshoppers' avoidance of the dropping can be considered a predator avoidance behavior. This avoidance behavior with the presence of a scent cue could have applications in efforts to deter grasshoppers or modify their behavior in areas where they are unwanted or harmful to vegetation. However, it is important to note that grasshoppers must balance the trade-off between

avoiding predators, accessing greater food availability and quality, and finding warmer conditions (Pitt, 1999). To do this they utilize different microhabitats by moving a few meters or even centimeters in 3D space, and depending on the balance of needs, risk, and availability, the avoidance behavior may not be as strong in certain conditions (Pitt, 1999). This means that more than just posing a predator risk is a factor in deterring grasshoppers, and that things such as resource availability, temperature, habitat structure, and diversity needed to be considered.

Initially, diversity metrics such as relative abundance and Shannon Index were intended to be assessed as well as overall density of the grasshoppers, however insufficient data was collected to do so. To collect this data, a sweep net was used to catch and identify grasshoppers in and around each ring, but more than half of all rings in this study had 0 grasshoppers caught, including rings with very high as well as lower densities as assessed by the pointer method. Often when sweeping the ring areas, the grasshoppers were observed to be quite good at escape before even getting near the net. A more aggressive or swift swing of the net may have achieved a higher capture rate, at least one more proportional to the densities found at each site, but that could not be done because that would have risked disturbing the ring placement. Since the rings were used more than once, hitting and moving the rings could have had an adverse effect on the results. Additionally the grasshopper's vertical position in the habitat may have affected their possibility of being captured by the net since grasshoppers are known to remain lower in the vegetation with the risk of avian predators (Pitt, 1999), and grasshoppers very low to the ground were more likely to be missed by the net.

If this project to be repeated or expanded on, a different way to collect individuals for diversity data may be necessary, or sweep nets could possibly be used if rings were only to be

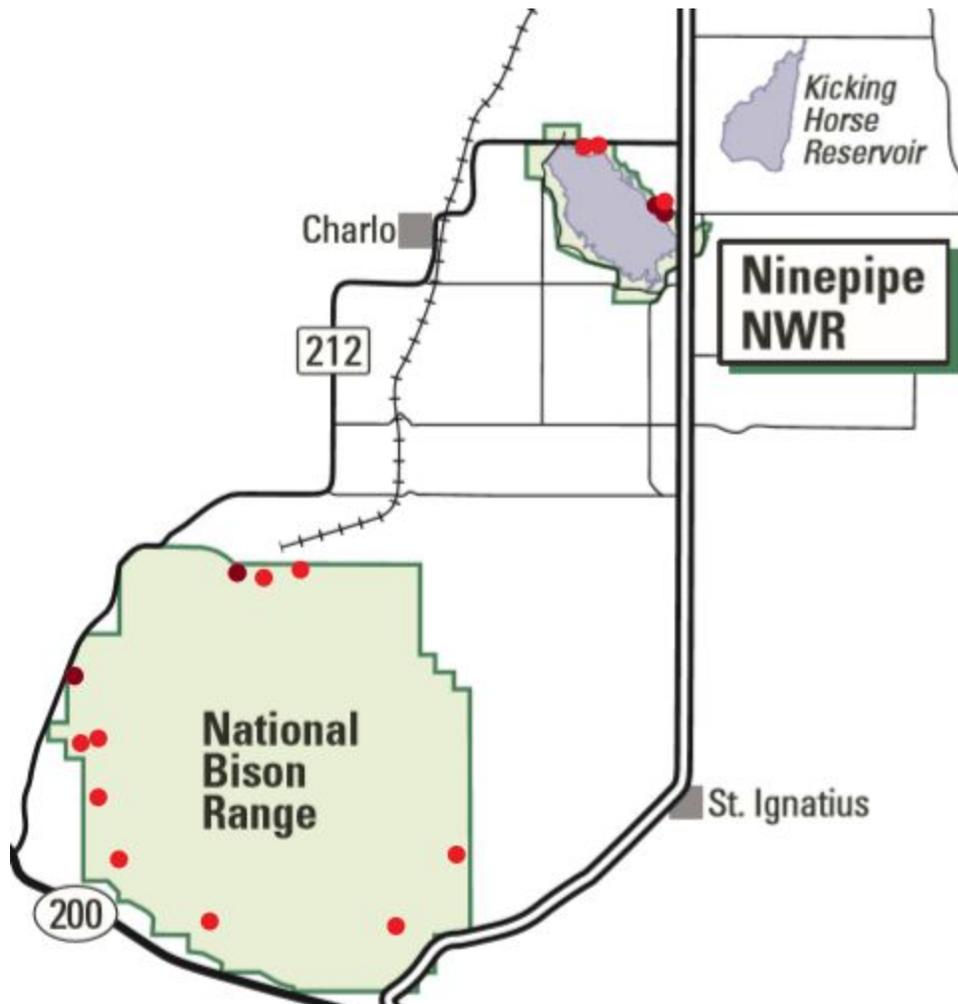
used once, allowing for a more vigorous sweeping. Any future research should assess diversity to see how things like grasshopper species richness and relative abundance are affected by the presence of predator waste. Grasshoppers diversity has been found to be affected by the presence and predation of birds, so assessing those metrics in response to cues such as bird droppings could be interesting. Belovsky and Slade (1993) found that bird predation reduced the abundance of large and small grasshopper species, but medium-sized grasshopper species became more abundant. They explain that since birds preferentially targeted larger grasshoppers, they produced indirect effects on competitive interactions between the grasshoppers of different sizes leading to those changes in relative abundance. In eastern Montana, a similar indirect effect was found in birds with size-selective predation on medium-bodied grasshoppers leading small-bodied grasshoppers to increase in relative abundance (Branson, 2005). These findings may suggest that certain sizes or species of grasshoppers may react differently to the presence of predatory bird droppings in an area, and this idea should be explored further.

Also, the collection of bird dropping could and should be done differently. Ideally, the bird poop used for this research would have been fresh from birds of a consistent known species that have recently eaten grasshoppers and are found near each site. Due to time and resource restrictions this was not possible for this research. Droppings were collected near the sites, but the age and source species was unknown. Additionally, it was unknown how recently, if at all, the birds that left the collected droppings last consumed grasshoppers. Droppings from birds that were recently fed grasshoppers would be ideal for this research based on the findings of Kortet and Hedrick (2004) that indicate that orthopterans, or at least crickets, only avoid waste from predators that have recently consumed the prey species. Most collections for this project were

likely from swallows, a known grasshopper predator, based on what birds were seen near the droppings collection areas and research sites, but this is not certain and the poops could be from a mix of bird species. It is important to recognise that even without using the ideal bird droppings, significance was still found between treatments. If this ideal collection can be achieved, the results could possibly see a greater difference than those found here.

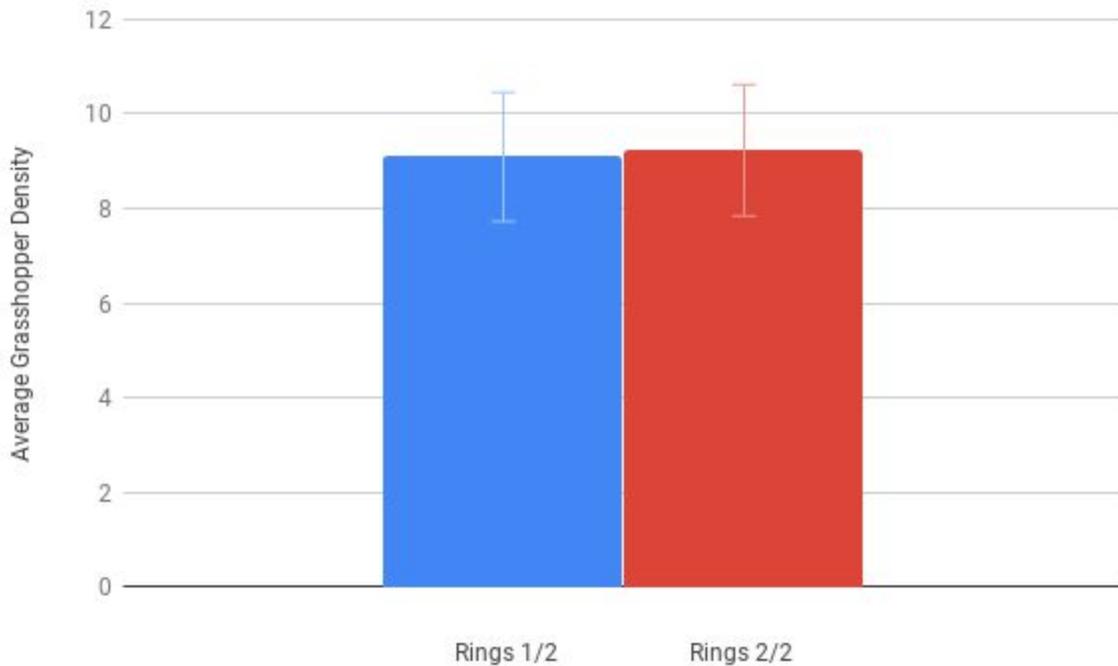
Even with less than ideal dropping collections and a failed diversity assessment, the results of this study did demonstrate a significant difference in grasshopper density with the presence of bird droppings, and these results are an important start to addressing the question: can predator excretions be used to reduce grasshopper herbivory and repel them from an area? These results supported the hypothesis that the density of grasshoppers in areas with bird droppings present would be less than that of other areas without. Grasshoppers were found in fewer numbers in areas where droppings were placed compared to similar areas without them present, indicating that grasshoppers were able to detect and respond to the droppings by avoiding the area. This means predator excretions can possibly in some way be used to reduce grasshopper herbivory and repel them from certain areas. These results, as well as any future findings that may stem from this, could have future implications in grasshopper deterrence efforts and the understanding of ecological and trophic dynamics in grassland ecosystems.

## FIGURES

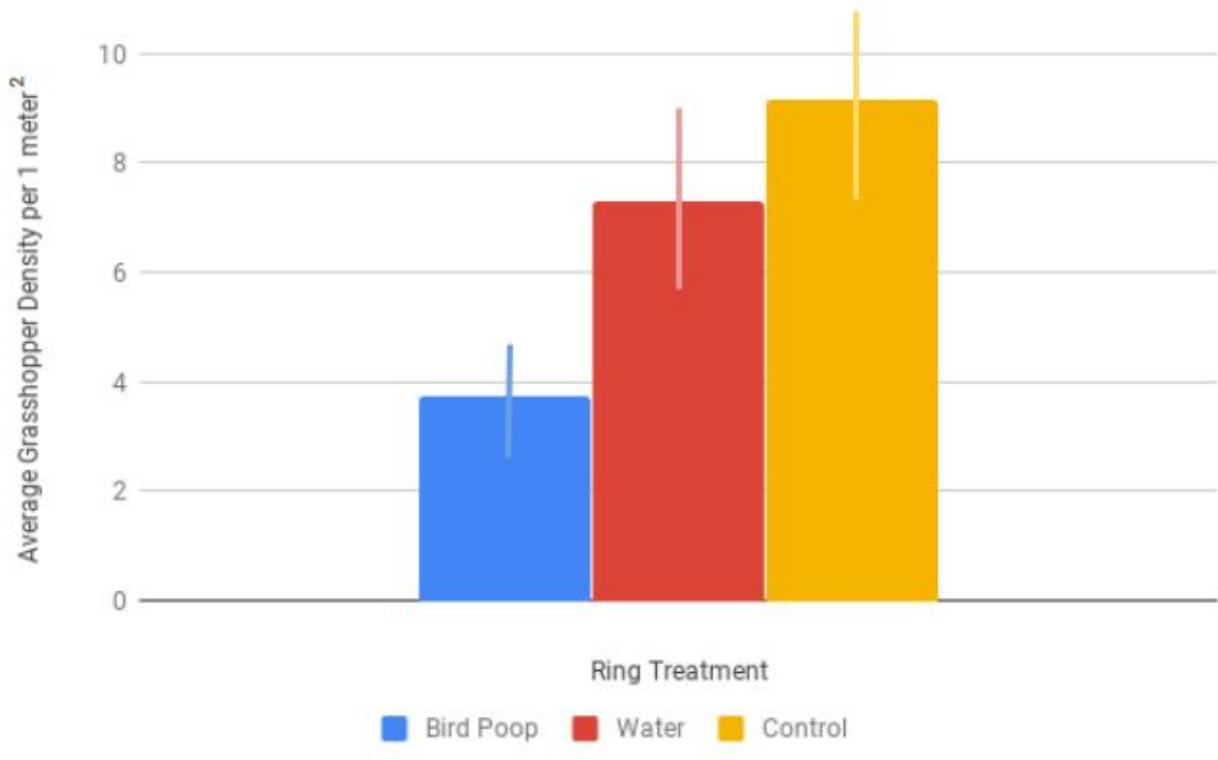


**Figure 1:** Map of research area indicating site locations with red points.

Bright red points indicate sites where one ring pair was used for data collection. Dark red points indicate sites with two ring pairs were used for data collection.



**Figure 2:** Average Grasshopper density of the empty rings from each pair. Density is in individuals per meter squared. The first rings of the pairs had an average density of 9.224 individuals/square meters and the second rings averaged a density of 9.086 individuals/square meters. A paired t-test shows there is no significant difference in average grasshopper densities between the rings of each pair (p-value = 0.8336), indicating that further assessments comparing rings in each pair can be done because they are significantly similar before treatments. Error bars represent standard deviation.



**Figure 3:** Average density of grasshoppers for each treatment of rings.

The average density of grasshoppers in the rings with bird poop treated cotton balls was 3.717 individuals/square meter. The average grasshopper density of the rings with water treated cotton balls was 7.296 individuals/square meter, and the average density of the empty control rings was 9.155 individuals/square meter. Error bars represent standard deviation.

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