

**Ecology of Fear: Behavioral Responses of Woodland Deer Mice (*Peromyscus
maniculatus*) to Direct and Indirect Predator Cues**

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Abstract

Mammals forage under different constraints that affect how much time and energy is spent gathering food. Fear of predation may be the most important type of constraint under which mammals forage. To test these constraints, I measured foraging preference of Woodland Deer Mice (*Peromyscus maniculatus gracilis*) on different substrate types (wet or dry leaves) along with different scents (mink urine and water), expecting mice to forage more often on wet leaves (a quiet substrate) with water as a scent. I found that *P. maniculatus* preferred foraging upon dry leaves with water as a scent. After further examination of foraging behavior, I discovered that mice on average handle seeds longer in trials treated with mink urine and less often in trials treated with water. These findings are important to understanding how rodents balance their energy budget and further support optimal foraging theory.

Introduction

Mammalian prey species forage under many different constraints (Orrock and Danielson 2009) of which predation risk may be the most important. Predation risk is frequently assessed by different types of cues, ranging from inherent or direct cues (i.e. predator scents) to non-inherent or indirect cues (i.e. forest cover; Pastro and Banks 2006). These cues can change the foraging behavior of mammalian species (Borgo et al. 2006, Fanson 2010). In many cases, indirect cues may have a more important effect than direct cues on prey species (Fanson 2010, Orrock and Danielson 2009, Orrock et al. 2004, Pusenius and Ostfeld 2002). Other studies show how direct cues, that stimulate olfactory nerves, can also change mammalian behavior (Borgo et al. 2006, Herman and Valone 2000).

Many of these studies have been conducted only in the field (Fanson 2010, Orrock and Danielson 2009, Orrock et al. 2004, Pusenius and Ostfeld 2002). Although field based studies offer a more “natural” environment for the system being studied, they contain shortcomings when measuring behavioral changes, especially the inability to control variables. Often times the olfactory sense of a human lacks in comparison to those of other mammals. This can lead to unknown scents to be present at a study site and skew results. Another shortcoming lies in the difficulty in studying small nocturnal species, especially rodents.

Rodents are a logical choice for researchers to use for studies of predation risk. They are extremely abundant, they are easier to handle and house than larger mammals, and there is less competition between trappers and hunters for rodents (Kurta 2003). However, rodents are elusive and nocturnal (Cramer 2014, Kurta 2003), which makes it difficult for researchers to observe rodents directly in the field. Non-invasive measures must be taken to gather unbiased and accurate data. On numerous occasions, giving-up-densities (GUDs) are used to measure the foraging behavior mammals (Bedoya-Perez et al. 2013). GUDs fall short because the number of animals visiting the area is often unknown and it is hard to estimate the actual number of individuals visiting the study site (Bedoya-Perez et al. 2013).

Frequently indirect cues, such as canopy and cloud coverage, are studied more often than direct cues in the field (Fanson 2010, Orrock and Danielson 2009, Orrock et al. 2004, Pusenius and Ostfeld 2002). Substrate type (i.e. dry leaves or wet leaves) is one indirect cue that has not been thoroughly studied. I examined how Woodland Deer Mice (*Peromyscus maniculatus gracilis*) forage on different substrate types in the presence of a

predator scent. *P. maniculatus* was chosen because it is an important seed predator and prey species for many different predator species (Cramer 2014, Kurta 2003). *P. maniculatus* also acts as a biological indicator of change for many different ecosystems due to its abundance all over the United States (Leis et al. 2008). Although it is known that *Peromyscus* respond to both direct and indirect cues, it is not certain how these cues might interact (Fanson 2010, Orrock and Danielson 2009). I hypothesize that *P. maniculatus* will not forage as often on a noisier substrate (dry leaves) in the presence of a predator scent (Mink urine, *Mustela vison*). Mink urine was used because they are natural predators of *P. maniculatus* in the region.

Methods and Materials

Study Site

The University of Notre Dame Environmental Research Center (UNDERC) comprises 3035 hectares on the border of Wisconsin and the Upper Peninsula of Michigan (Cramer 2014). Many lakes and bogs fall within the area and account for a surface area of 540 hectares. The elevation is between 500 and 520 meters. The habitat of the region is considered a secondary growth northern hardwood deciduous forest. The property has been closed off to the public for the past 100 years. Logging on the property still continues to this day in selective areas, but clear-cut logging has since ceased (M. J. Cramer, personal communication).

Experimental Design

Over the course of May –July 2015, I captured *P. maniculatus* in specified 60x60 m² grids, as part of a larger population study. Female mice that were either lactating or pregnant were not used in any trial and mice that weighed less than 15 grams were

excluded as well. This was done in an effort to reduce bias because pregnant females would consume more seeds and smaller mice do not require as much to eat as average adults. All handling of the mice followed the guidelines provided by the Institutional Animal Care and Use Committee at the University of Notre Dame (protocol number 14-04-1722).

To test the interaction between substrate and scent on foraging behavior, I used a large aquarium as an arena. Inside of the aquarium, I placed one of two substrate types, dry or wet matted paper leaves. I used paper leaves to reduce the unknown variable of scent on fallen leaves gathered from the surrounding area. In an effort to create a more natural environment, I placed sand on the bottom of the arena, beneath the leaves. The dry leaf substrate was dried out to increase the similarities to actual leaves. The wet matted leaf substrate was soaked in water to try to produce a realistic simulation of wet forest litter. For each substrate type I used two scents, *M. vison* urine or water, as a control. One milliliter of scent was placed on a cotton ball inside of a film canister with holes. The film canister was attached to a plastic base inside the arena. Each substrate (wet or dry leaves) was combined with one of the two scents (water or mink urine), creating four different treatments, and was replicated seven times.

Red maple seeds (*Accer rubrum*) were placed in the arena under the leaves. Ten grams of dried *A. rubrum* seeds were used in each trial. Each trial lasted 30 minutes and was filmed remotely by a video camera (SONY DCR-SR45) equipped with an infrared sensor. Trials were conducted no earlier than 10 PM and each mouse had its food removed five hours prior to the trial. The mice were given a two-minute acclimatization period inside of the arena before the trial began. After the trial ended, the seeds were

separated from the sand, dried, and weighed. Every video was watched afterwards and I used the program JWatcher to quantify the behavior of the mice (Blumstein and Daniel 2007).

Statistical Analyses

I used a two-way analysis of variance (ANOVA) to measure the interaction between substrate and scent with the difference in weight of seeds before and after the trial. I used another two-way ANOVA to measure the interaction between substrate and scent with average handling time per seed. I used the Shapiro-Wilk test to determine if my data were normally distributed. Because of deviations from normality, a logarithmic transformation was performed on the average handling time per seed data set. All statistics are reported as means \pm SE.

Results

A total of 28 individual mice were used. Each mouse experienced one of the four treatments: wet or dry leaves with either mink urine or water. Their foraging activity was measured in amount of seeds eaten. *P. maniculatus* did not respond to either substrate or scent individually ($F_{[1,24]}=3.73$, $P = 0.065$; $F_{[1,24]} = 4.15$, $P = 0.052$, respectively). However, there was a significant interaction between both substrate and scent ($F_{[1,24]} = 7.56$, $P = 0.011$; Figure 1). *P. maniculatus* showed a stronger foraging preference towards the dry leaf and water scent treatment (0.535 grams \pm 0.068) compared to the other treatments of dry leaves and mink urine (0.239 grams \pm 0.053), wet leaves and water (0.245 grams \pm 0.068) or wet leaves and mink urine (0.289 grams \pm 0.063).

In addition to measuring foraging preference, I quantified the behavior of the mice for each trial. Specifically, I quantified the average amount of time, in seconds, each

mouse spent handling seeds. The data was not normal (Shapiro-Wilk test: $W = 0.905$, $P = 0.014$). I performed a logarithmic transformation and another Shapiro-Wilk test to retest for normality again and found that my data were indeed normal ($W = 0.979$, $P = 0.824$). A two-way ANOVA found no significant interaction between substrate and scent in regards to the average time spent handling seeds ($F_{[1,24]} = 0.367$, $P = 0.55$). There was not a significant difference between substrate types ($F_{[1,24]} = 0.324$, $P = 0.574$; Figure 2), but there was a significant effect of scent treatment ($F_{[1,24]} = 6.24$, $P = 0.019$; Figure 3). Mice in trials that were treated with mink urine had a higher average handling time per seed (46.927 seconds \pm 6.203). Alternatively, mice in trials treated with water had a lower handling time (33.95 seconds \pm 4.991).

Discussion

P. maniculatus showed a stronger preference for feeding in trials that were treated with water and dry leaves compared to the other trials. This was contrary from my original hypothesis. It was not surprising that the mice ate more in trials with water, but it was surprising that they ate more in a louder substrate. One explanation as to why the mice chose to use the treatment with a louder substrate might arise from the lack of a perceived predator risk. In trials with mink scent, there was significant difference in the amount of seeds eaten (Figure 1). Although the data indicates that direct cues (mink urine) do affect how mice forage, that is only part of the story. It does not explain why the mice did not eat as many seeds during the wet leaves and water treatment.

The second explanation for this phenomenon is connected to optimal foraging theory: how organisms balance energy costs in terms of time when searching for food (Higginson and Houston 2015). Mice in trials that contained the wet leaves substrate

might have had a harder time accessing the seeds under the wet leaves and decided to eat seeds that were easily found. Alternatively, the seeds were not as desirable when wet. However, the latter would not follow the optimal foraging theory model because if mice were in need of energy, they would consume seeds, regardless of desirability. In any case, it is not totally clear why the mice ate less in trials containing wet leaves. What is clear is that neither substrates nor scents entirely explain the results. It is the combination of both treatments, direct and indirect cues, working synergistically, that changes foraging activity in *P. maniculatus*.

These findings are contradictory to recent literature findings. Studies indicate that indirect cues play a stronger role than direct cues in how rodents forage (Fanson 2010, Orrock and Danielson 2009, Orrock et al. 2004, Pusenius and Ostfeld 2002). In this case, the indirect cue (substrate type) was not strong enough on its own to change how the mice foraged. Substrate may not play a large enough role to change how rodents, in general, forage. More research should be done on different substrate types in an effort to determine if substrate plays a larger part in foraging behavior in *Peromyscus* and other rodent species

P. maniculatus had an interesting behavioral response to the different scent treatments when handling seeds. On average, mice handled seeds longer in trials treated with mink urine and less in trials treated with water (Figure 3). Mice in trials treated with mink urine perceived a potential predator in the area and took longer to eat the seeds. This increase in average handling time per seed is due to the mouse allocating more time to vigilance while handling. On the other hand, mice in trials treated with water did not perceive as high of a risk when handling seeds and spent less time on each seed. Baker et

al. (2011) supports this hypothesis and found that granivorous species were more vigilant during handling and incurred a time-cost.

It is evident that a predatory scent affects the foraging behavior of *P. maniculatus*. Although it has already been shown that predatory scents can alter foraging behavior in rodents (Sunyer et al. 2013), not many studies decipher the alteration in behavior. Researchers should further investigate the behavioral responses of *Peromyscus* species and other rodents to predatory scents.

In conclusion, these findings highlight important behaviors *Peromyscus* species have towards direct and indirect cues while foraging. In particular, it shows how *P. maniculatus* rationalizes energy trade-offs while foraging. Furthering our understanding of how *P. maniculatus* balances its energy budget within optimal foraging theory sheds light on how other similar species do the same. Species that disperse and cache seeds are also important for forest structure and composition (Cousens et al. 2010). Therefore it is important to understand their role in the ecosystem and how that might change as species diversity decreases and homogenization of species increases (Rahel 2000).

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Figures

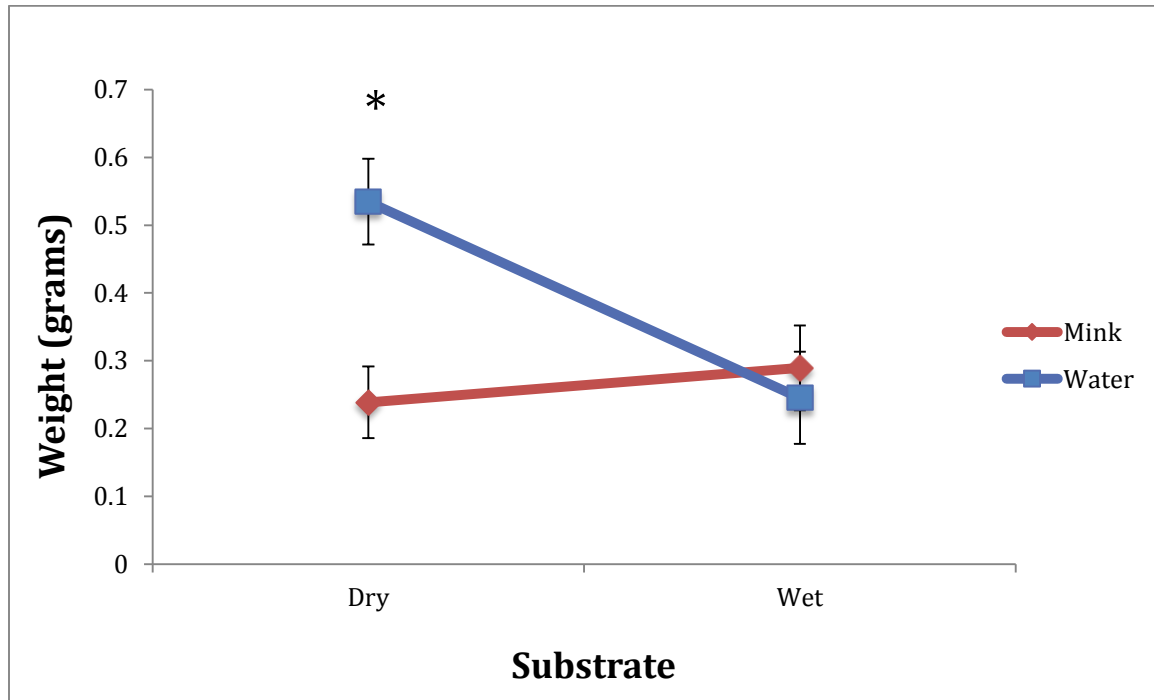


Figure 1. Interaction between substrate and scent on foraging activity (mass of red maple seeds eaten) of *Peromyscus maniculatus*. Response to scent was only evident in the dry leaf substrate (ANOVA: $F_{[1,24]} = 7.56$, $P = 0.011$). Whereas the other combined treatments had no interaction at all. Neither scents nor substrates factors were statistically significant.

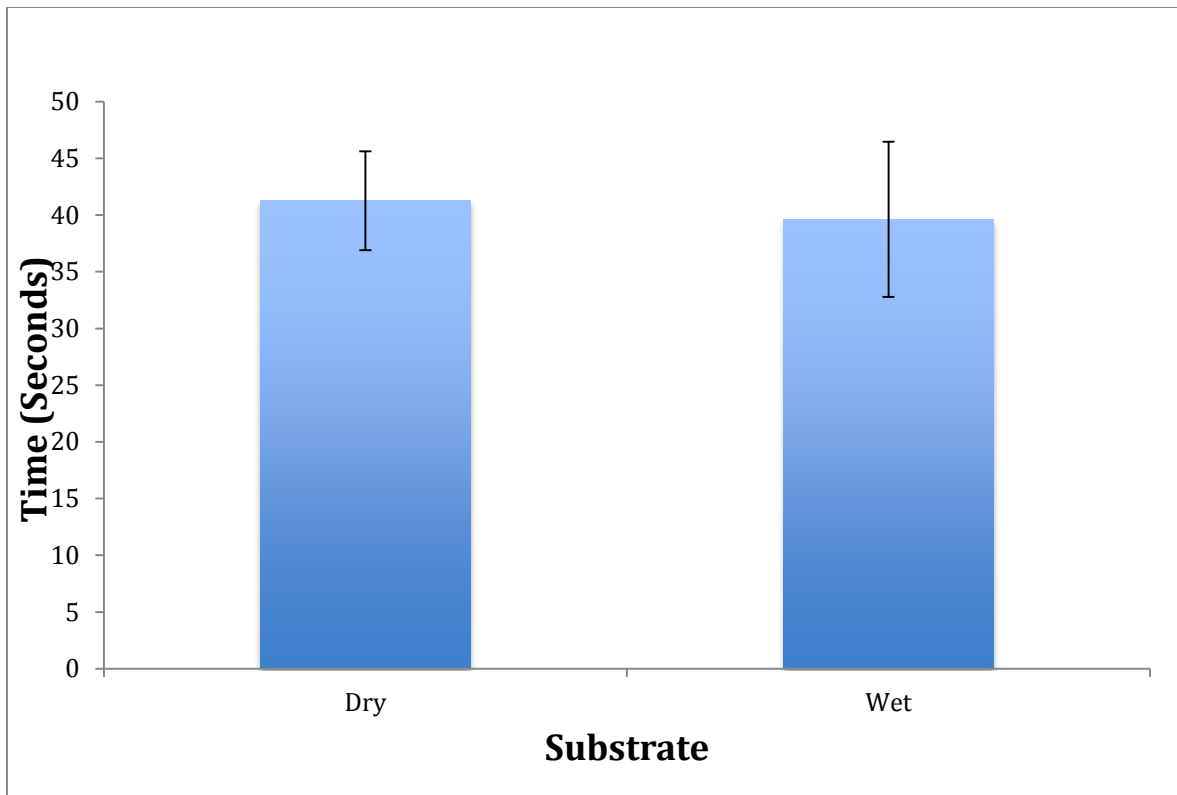


Figure 2. Average handling time per seed, in seconds, in different substrate types. There was no significant difference between substrate types (ANOVA: $F_{[1,24]} = 0.324$, $P = 0.574$). The dry substrate had a higher mean (41.249 seconds ± 4.359) compared to that of the wet substrate (39.637 ± 6.834).

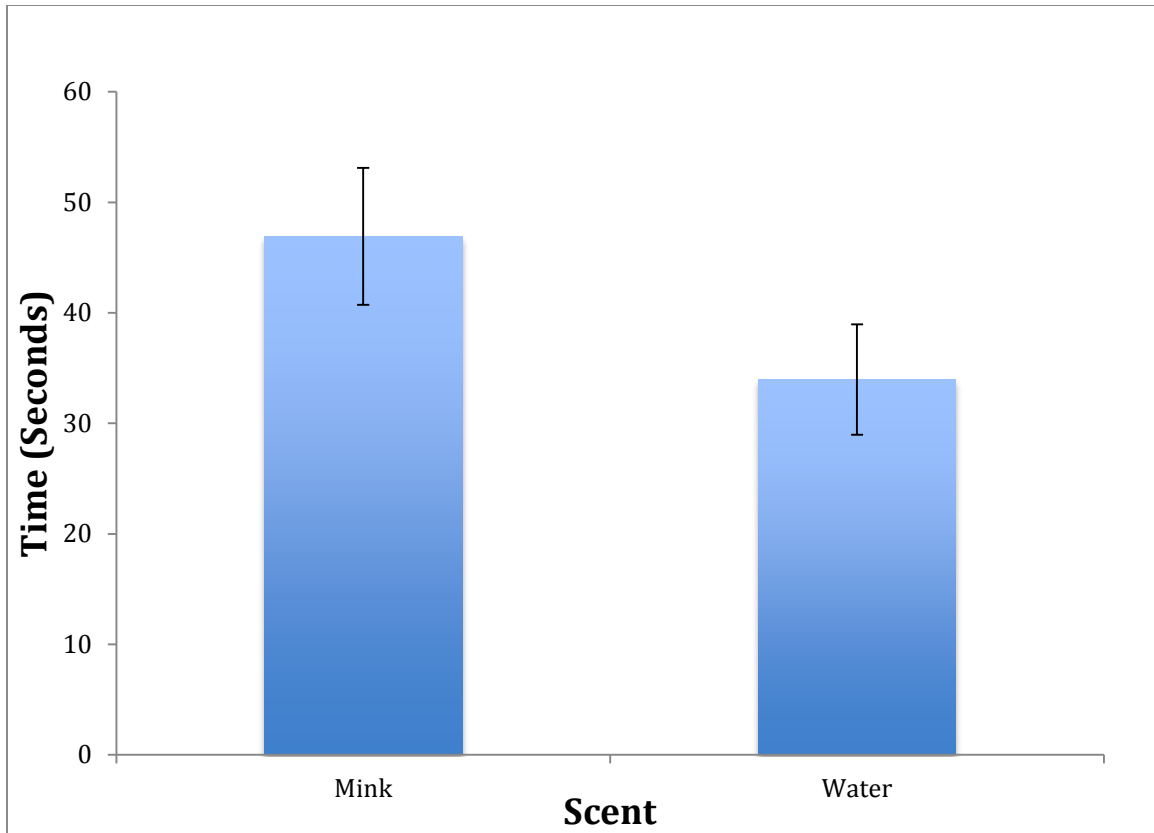


Figure 3. The relationship between average handling time per seed, in seconds, and the treatment scent. There was a significant difference between scent types (ANOVA: $F_{[1,24]} = 6.24$, $P = 0.019$). The average handling time per seed for the mink urine treatment (46.927 seconds \pm 6.203) was higher compared to that of the water treatment (33.95 seconds \pm 4.991).