INFLUENCE OF PREDATION ON FEEDING AND GIVING-UP DENSITY OF FOREST RODENTS

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by

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I. INTRODUCTION

Risk and reward are two factors that play a key role in determining the foraging activities and diets of woodland animals (Halliday and Morris 2013, Banasiak and Shrader 2016, Bleicher et al. 2020). Animals are often forced to adapt to the trade-off of risking safety for some reward, typically food (Brown 1988, Makin et al. 2012). While daunting, animals must balance the need to forage while exposing themselves to predation (Laundré et al. 2010, 2014). Successful foraging determines the likelihood of survival of the individual organism and its reproductive potential, as growth and food consumption allow for investment in reproduction. The delicate balance of feeding and the back-and-forth nature of predator-prey relationships is important for many reasons, including the health and productivity of the ecosystem in question. Predators determine the health of the ecosystem because they are more likely to prey on animals that are weak, injured, or in some way more vulnerable than others (Sinclair and Arcese 1995). Even more importantly, they play a role in keeping populations of their prey species in check as well, preventing overgrazing in these ecosystems.

The act of foraging is vital to every animal. Whether herbivore or carnivore, every animal must be able to sustain itself, and, in some cases, its young. Therefore, foraging behaviors should be finely tuned to allow the animal to collect enough food for itself and its offspring (if it has them). It is also very important for adults to pass these behaviors on to their offspring so that they may also be successful. If an animal forages in a specific way, the offspring are likely to forage in the same manner (Pyke 1984). Thus, it is important to gain an understanding of foraging strategies and behaviors to understand how they meet the needs of the animals and their offspring. In order to employ the best strategies for finding food, animals learn to forage optimally relative to the availability of food and the risks (e.g., from predation) associated with foraging successfully. Understanding these behaviors has become a field of study in and of itself, known as 'optimal foraging theory'. Optimal foraging theory assumes that the fitness of an animal is linked with its foraging behavior (Pyke et al. 1977). Therefore, foraging is directly linked to natural selection.

One way that animals can employ optimal foraging theory is by maximizing the energy gained, while minimizing the energy required to get it (Pyke et al. 1977). An animal's time and energy are limiting resources, and therefore, they must be used wisely. One way to determine if time and energy are being allocated optimally is by determining the net energy gain the animal receives. A general rule is that an animal should spend more time in a certain patch as long as the resulting gain exceeds the loss. Therefore, when the loss exceeds the gain, animals should no longer allocate time to feeding in that patch (MacArthur and Pianka 1966).

Ultimately, there are many factors that influence how long an animal spends foraging in a given patch and how efficiently they can forage. One general idea is that in order to ensure their own safety, animals must protect themselves while simultaneously collecting food to sustain themselves. Safety/protective measures may include hypervigilance, apprehension with respect to a new food source, and choosing among multiple food sources and food patches with respect to perceived predation risk. In addition, any apprehension that an animal has to a food source can negatively affect the time it takes to forage and the number of food items collected, since the animal's attention will be allocated towards potential predators and not towards foraging behavior (Dall et al. 2001). Using these behaviors in making these decisions, in addition to stress and paranoia, can exhaust an animal, resulting in increased energy requirements for survival. An important question in these types of studies then is how animals can increase their net energy gain while allocating energy to manage threats such as predation risk, and how they make decisions relative to time spent feeding.

Prey animals exhibit a broad range of different behaviors to assess/examine the level of threat around them. Because vigilance is exhibited when animals are visually scanning their surroundings, it can be an easy behavior to document (Caro 1987). This can be seen in many animals as an anti-predatory behavior to ensure safety for both the animal itself and the animal's offspring, if it has them (Caro 1987). Vigilance and foraging are the two sides to the key trade-off/balancing act facing many animals. Prey must choose an optimal level of vigilance where they are still able to forage efficiently while still being apprehensive and aware of their environment and potential predators (Brown et al. 1999). Vigilance can be broken down into two categories. 'Routine vigilance' is exhibited when the animal is regularly monitoring its surroundings, whereas 'induced vigilance' is exhibited when an animal is responding to a stimulus (Blanchard and Fritz 2007). Vigilance is important in the detection of potential predators, however, prey are also able to detect and respond to cues directly from the predator, including

visual, auditory, and olfactory cues. The ability to detect and respond effectively to these cues greatly increases survival, with responses including freezing in place, increasing vigilance, and/or fleeing to a refuge (Thorson et al. 1998). Rodents can display these responses, often enhancing their survival; for example, fox squirrels (*Sciurus niger*) can directly respond to olfactory cues of a predator, while thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*) will respond to visual cues, like a plastic owl placed in their environment (Thorson et al. 1998). Ultimately, the ability of prey to detect and respond to cues from a potential predator, even if it means expending more energy, greatly improves their chance of survival.

Vigilance can be a very species-specific behavior. Depending upon feeding preferences, some animals may have more opportunities to be vigilant. Certain herbivores may have more vigilance opportunities because they are able to observe their surroundings while consuming their food. For example, the American bison (*Bison bison*) and elk (*Cervus canadensis*) can be vigilant (head up, scanning the surroundings) while chewing vegetation (Fortin et al. 2004). In contrast, other species will feed with their head down, which is clearly a much more vulnerable position with limited potential for vigilance (other than using sound-based or olfactory cues). Lastly, routine vigilance does not impact foraging time in the same way that induced vigilance does. Induced vigilance can be much more costly, disrupting feeding behavior, ingestion of food, and forcing an animal to react to a stimulus, no matter the phase of feeding (Blanchard and Fritz 2007).

In addition to behavioral studies focused on observing animal responses to their foraging environments, a useful way to study animal foraging is with giving-up density

studies (Brown 1988). 'Giving-up density' (GUD) refers to the density of resources in a patch at which the forager stops foraging, likely as a result of increasing costs (e.g., predation risk, low food density or potential energy gain). It can often provide more information about the forager than simply the amount of food they consume/harvest (Brown 1988). In general, the giving-up density of a forager should correspond to the harvest rate that balances metabolic costs of foraging, the risk of predation, and the rate of missed opportunities that the forager experiences by remaining in a given patch (Brown 1988). This concept emphasizes the idea that foragers will have a higher givingup density when they engage in activities like hypervigilance. In other words, they are more likely to give up foraging in a given patch due to the threat of predation. A high giving-up density indicates that the food patch has low value to the forager with respect to the costs of foraging there (Druce et al. 2009). For example, klipspringers (*Oreotragus oreotragus*, a small African antelope) exhibit high giving-up-densities in areas close to human-placed drainage lines and low giving-up densities in areas containing rocks, suggesting that klipspringers perceive greater predation risk when farther from the protective influence of the rocks (Druce et al. 2009). Likewise, white-tailed deer (Odocoileus virginianus) show higher giving-up-densities in areas where they may sink in deep snow, with deeper snow increasing the giving-up density. These deer determine the habitat to be riskier because they are less likely to be able flee from predators when the snow cannot support them and impedes their movement (Riceucau et al. 2009). Therefore, by using giving-up density as a tool, it is possible to analyze the relative risk of predation for a given area.

Measuring giving-up densities for certain species (e.g., rodents, such as squirrels and chipmunks) can be more complicated because they live in and use many different habitats across different landscapes. For example, while determining the giving-up density for a squirrel in a forested habitat may help establish the perceived predation risk of that habitat, it would give no insight into the giving-up density of squirrels living in a more urban environment. In fact, gray squirrels (*Sciurus carolinensis*) in high-density urban areas exhibit a lower giving-up density (lower perceived predation risk) than squirrels living in rural environments with less anthropogenic activity (Bowers and Breland 1996). This pattern could be the result of perceived predation risk, acclimation to human disturbance (higher in urban habitats), or perhaps, rural squirrels had more profitable food items/patches nearby (Bowers and Breland 1996).

As an inhabitant of urban areas, it is crucial that urban squirrels adapt their vigilance behaviors to their unique habitat. If squirrels constantly view humans as a predator, they would expend all of their energy on such behaviors, which would not leave time or energy for foraging and reproduction (McCleery 2009). Therefore, they may be less likely to leave a food patch in an urban environment because they are acclimated to (and not as responsive to) nearby humans and their activity, whereas squirrels living in a rural environment use anti-predator behaviors more frequently and are more likely to leave a patch due to risks they face. Therefore, when studying the foraging behavior of rodents, particularly those adapted to urban areas, it is important to consider the foraging activity and behavior of animals in each habitat separately.

Another consideration for rodents and other animals is the presence of edges between habitat types and how they influence foraging behaviors and decisions when different habitats are side-by-side. In these circumstances, the relative predation risk for a given area can be best studied by breaking up the overall foraging landscape and habitats into separate components. A range of biotic and abiotic factors contribute to risks and rewards in different habitats within a landscape, including the abundance and types of predators and prey that are present, habitat structure (such as foliage type, shape, height, diversity), and foraging opportunities (i.e., the abundance, diversity, and availability of food resources), and it is important to take these into account in a habitat-specific manner.

Further, the relative success of prey and their predators plays into habitat-specific risk as well. It is conceivable that in a given landscape, there will be habitat-driven variability in predator success and in prey ability to detect predators. This is because all animals have evolved and adapted differently to their surroundings, and certain animals are more skillful under certain circumstances than others. Therefore, the risk of predation can change relative to landscape diversity, and the abundance and types of prey and predators (Laundré et al. 2010). With respect to this idea, prey animals that are typically arboreal may spend less time on the ground, even if they are foraging, in order to minimize their risk of predation, demonstrating a clear preference relative to components of the landscape they will use. Similarly, common brushtail possums (*Trichosurus vulpecula*) feed more at above-ground feeders when compared to on-ground feeders if given the choice, due to increased risk associated with ground-level foraging (Mella et al.

2014). Therefore, by studying giving-up densities in different habitats, particularly when they are adjacent to each other and the animal in question can/will use both habitats, the perceived risk of predation may be calculated more accurately, and we may gain greater insight into how foragers perceive different habitats in a landscape.

In the same way, the amount and quality of food can influence the giving-up density and optimal foraging strategies of an animal, as it assesses the trade-off between food availability/quality and foraging opportunities. For example, oribi (*Ourebia ourebi*; a small African antelope) prefer to forage in grasslands rather than woodlands due to differences in the perceived predation risk associated with these two habitats (Stears and Shrader 2015). However, as food availability in the woodlands increased, oribi switched to feeding there (i.e., in the riskier habitat; Stears and Shrader, 2015). That said, further increases in food availability could not persuade the oribi to forage near effective predator ambush sites, suggesting that the risk of predation was far too high, and the benefits of the rich food patch did not outweigh the risks (Stears and Shrader 2015). Likewise, the quality of available food can influence the trade-off between foraging and predation risk. Nubian ibex (*Capra nubiana*) choose high quality food with less tannic acid, a compound that negatively affects protein digestion, particularly when they have access to escape routes that will offset the threat of predation (Hochman and Kotler 2006). In addition, the presence of a water source can alter the quality of an available food patch. In the same study, Nubian ibex foraged more when a water source was close to the food patch, suggesting that water intake may be just as important as food intake in their patch use decisions (Hochman and Kotler 2006). Ultimately, the quality of a food

patch is determined by many factors, including food quantity, food quality, and water availability. All of these can influence the giving-up density of a food patch, and they can be used by foragers to off-set perceived risks of predation.

Ultimately, one must also consider spatial and temporal scale in these types of interactions as well (Levin 1992). Scales associated with the foraging behavior and landscape, as well as predatory behavior and the response to it, will drive interpretation of these relationships and studies that attempt to understand them. It is hoped that my study will also provide insight into this issue as well.

In the first part of this study, I used three experiments to investigate the effects of perceived predation risk on habitat-specific foraging patterns of typical temperate forest animals, particularly rodents [i.e., gray squirrels (*Sciurus carolinensis*), fox squirrels (*Sciurus niger*), and Eastern chipmunks (*Tamias stratus*)]. Foraging activity was compared between adjacent habitats (i.e., forested and open habitats) in two different landscapes. The two landscapes differed in the degree of anthropogenic influence and activity they typically experience. The first site was a typical forested site, with very low anthropogenic influence or disturbance, while the other site was a more disturbed site (on Kent State University's campus) and much more directly affected by human activities. At each of these sites, I was able to investigate and compare foraging patterns in forested and open habitats adjacent to each other, which I assumed to have different levels of predation risk due to differences in cover and vegetation. To determine differences in foraging patterns, I used giving-up density to assess the relative predation risk in these habitats at these sites.

The second part of my study was a single experiment focused on manipulating food availability and perceived predation risk in forested and open habitats at a single site (the more natural, forested site). In this experimental design, I had two levels of food availability (limited vs. high), and two levels of perceived risk (background/typical perceived risk vs. perceived risk enhanced with coyote urine) in two adjacent habitat patches (forested and open). As in the first experiments, I planned to use giving-up density to assess treatment effects on foraging patterns. However, we had to end this experiment 2-3 days after it started due to restrictions placed on activity and people gathering by the onset of the COVID-19 pandemic.

Using these experiments, I planned to test the following hypotheses regarding factors affecting foraging patterns and behaviors by forest animals, particularly rodents: Hypothesis 1 (H1): Squirrels and chipmunks prefer to forage in forested habitat relative to open habitat patches, and thus, have lower giving-up densities in the forest, due to higher levels of cover (protection from predators) in the forested habitat. Hypothesis 2 (H2): Squirrels and chipmunks will have higher giving-up densities at the forested site with less human disturbance relative to the campus site, due to animal acclimation to high levels of human activity on campus (i.e., they will be less skittish and show lower levels of vigilance).

Hypothesis 3 (H3): High food availability will lead to lower giving-up densities relative to limited food availability regardless of habitat, but increased perceived predation risk (due to coyote urine) will reduce effects of food availability. Hypothesis 1 and 2 were addressed by the first three experiments, while Hypothesis 3 would have been addressed by the final experiment (for which no data was collected as it was ended early).

II. METHODS

Study Sites

Two sites were used in this study. The first study site was near the Allerton Sports Complex located on the south end of the Kent State University campus (KSU; Figure 1). The eastern side of the complex contains a wooded area that extends off-campus. The edge of the woods provided an ideal location for this study as it allowed wooded forest habitat to be directly adjacent to open mowed grassy habitat for placement of treatment bins in both habitats (described below). This location was chosen for its close proximity to human disturbance and activity as the Allerton Sports Complex is located only 600 m from the main Kent State University Campus. In addition, this is the site of a frequently used parking lot and university-sanctioned intramural sports. Clearly, potential foragers in this habitat have had the potential to become habituated to anthropogenic activity, and thus, might have altered their behavioral patterns relative to a more natural site.

The second study site was Jennings' Woods, a 74-acre property owned by Kent State University, located near Ravenna, Ohio (Figure 1). The different habitats located at this site, including forests, meadows, floodplains, and wetlands, provided a much more diverse and distinct landscape when compared with the on-campus site at Kent State University. More importantly, with respect to the overall design of the experiment, there



Figure 1. Study Sites for All Experiments. Left Panel: Allerton Sports Complex, Kent State University campus. Right Panel: Jennings' Woods.

is a gas pipeline right-of-way that is mowed annually (and resembles an old-field habitat, with diverse vegetation), running through this forest ecosystem. This right-of-way provides a forest edge that is similar to the forest edge found on Kent State's campus. It is important to note that there is very little human activity and interference at this site, thus, providing a more undisturbed habitat for the animals.

Focal Organisms

Originally, the focal organisms for this study were common forest rodents: gray squirrels (all color morphs), fox squirrels, and Eastern chipmunks. I chose these organisms because they are very active, reliable foragers that are also common prey for a variety of ground-based [coyotes (*Canis latrans*), red foxes (*Vulpes vulpes*), and feral cats (*Felis catus*)] and avian (hawks and owls) predators. However, as will be presented below, many other organisms visited the treatment bins as well, including raccoons (*Procyon lotor*), white-tailed deer, Virginia opossum (*Didelphis virginiana*), white-footed mouse (*Peromyscus leucopus*), and various bird species.

Design of Experiments in this Study

Overall, there were four experiments run during this study. Of these four experiments, only the first three were completed, as the fourth experiment was ended prematurely due to the COVID-19 pandemic. In experiments of this sort, there is often some acclimation period where study animals are given the chance to encounter and become acclimated to the experimental set-up before actual data are collected. However, brief, preliminary trials (conducted in the Cunningham Hall woodlot) demonstrated little need for acclimation as gray squirrels and chipmunks were readily approaching the bin within hours. Further, data from the first sampling period in the experiments described below did not appear to be substantively different from data collected during other sampling periods.

Experiment 1 (15-23 December 2019) and Experiment 2 (2-10 January 2020) shared the same overall design with two treatment types: Habitat (Forest vs. Open) and Site (Jennings' Woods vs. KSU campus). At each site, five clear plastic bins (44.5 cm long x 30.5 cm wide x 18 cm deep) were placed in the open habitat and five bins were placed in the forest habitat, for a total of 10 bins (20 bins total across two sites). The bins were placed in pairs, one bin in the open habitat and one bin opposite it in the forested habitat, each approximately 3 m away from the tree line into their respective habitats. Each pair of bins was placed approximately 50 m apart along the forest edge (Figure 2).

At the start of a given experiment, each bin was filled with 500 oil seeds (from the sunflower *Helianthus annuus*) in order to attract squirrels and chipmunks. The seeds were counted instead of weighed in order to increase accuracy when collecting the seeds after each sampling period. A large rock (4-5" diameter) was added to each bin for two purposes: 1) as an obstacle to increase foraging difficulty (and the costs associated with foraging in the bin) and 2) to weigh down the bins, reducing the likelihood of being tipped or blown over. Following the initial setup of the experiment, sampling occurred every other day, with all remaining seeds being removed for counting and 500 new oil seeds being added to each bin in order to keep attracting animals. Seeds were sampled

from bins four times during the course of each experiment (for a total of eight days). Seeds removed on each sampling date were counted for later calculation of bin- and location-specific giving-up densities.



Figure 2: Diagram of Experimental Setup of Experiments 1, 2, & 3.

The design of Experiment 3 (7-15 March 2020) was nearly identical to that of experiments 1 and 2, with two important differences. First, 10 black plastic tubes (~5" long, 0.5" diameter) were added to each bin as obstacles to increase foraging difficulty (and time required to forage), along with the large rock that was already present. Second, in addition to the bins, six game cameras were set at each site, with three cameras assigned to randomly chosen bins in the forested habitat and three cameras assigned to bins in the open habitat (for a total of 12 cameras across the two sites) in order to record foraging activity and behavior of the animals. These cameras were motion-activated and

programmed to take a single still photograph and a 20 sec video every time the sensor was triggered. As in the previous two experiments, seeds removed on each sampling date were counted for later calculation of bin-specific giving-up densities.

The final (and fourth) experiment was also very similar to the design of the earlier experiments. However, it was only going to be conducted at Jennings' Woods (the natural site with limited human disturbance). Habitat type (Forest vs. Open) was still part of the experiment, however, two additional factors were added to the experimental design. First, a predation risk treatment was added, with two levels: normal/unmanipulated perceived predation risk (requiring routine vigilance) vs. enhanced perceived predation risk (requiring induced vigilance). Second, a food availability treatment was added with two levels: limited food availability and high food availability. For this experiment, 40 bins were placed along the gas powerline right-of-way using the same pattern as in previous experiments, with paired bins in the Open and Forested habitats. Each bin pair was 3 m from the tree line (forest edge) into their respective habitats, with each pair approximately 50 m apart along the right-of-way. The 40 bins were divided into two groups along the right-of-way, with 20 bins in the front half of the site (near the road) and 20 bins in the back half of the site. The 20 bins located in the back half of the site received the enhanced perceived predation risk treatment. Coyote urine was sprayed at each corner of the bin so that the animals could smell it no matter how they approached the bin and to signal the presence of a potential predator. In contrast, the 20 bins in the front half were not treated with urine (and were representative of normal perceived predation risk). The predation treatments were separated in this way

to prevent the 'very ripe' coyote urine in the enhanced treatment from altering foraging behaviors in bins that were part of the normal perceived predation risk treatment.

Across all locations, each bin was randomly assigned a food availability treatment of either 100 g of oil seeds (limited food availability) or 500 g of oil seeds (high food availability), so that there were 10 bins of each feeding treatment in each habitat at the site. In each half of the site (front vs. back), there were five bins in each habitat with limited food and five bins with high food. As in Experiment 3, the plan was to use game cameras to investigate foraging behavior, with eight game cameras in each group of 20 bins with four cameras focused on randomly chosen bins in the open habitat, and four cameras focused on bins in the forested habitat. Unfortunately, as noted before, this experiment was ended after 2-3 days due to COVID-19 and no data was collected.

Game Camera Analysis

For Experiment 3, camera cards were removed from the game cameras and photographs and videos were downloaded for analysis. Camera-specific data (from Forest and Open habitats at both sites) was recorded by sampling period. Each video was viewed/analyzed and the following basic data was collected: Site, Habitat, Time of day, and foraging species. For each video, a range of behavioral data was also recorded, including inspection distance (within or outside of one bin length from the bin), lean-over (did the animal lean over the bin edge to inspect contents), tail-pumping (demonstrating nervousness or agitation), vigilance behavior (alertness: Low, Moderate, High), did the animal jump into bin to feed, or did it feed from the bin edge. Upon watching videos, it became clear that non-rodent foragers were feeding in both habitats at both sites. Thus, non-rodent foragers were also identified and recorded as part of the data set, and it was noted as to whether or not they were feeding and/or exhibiting vigilance behavior.

Statistical Analysis

Other GUD studies have used a range of approaches to data analysis. In some studies, they used each sampling period as an individual trial, and in others, they used some sort of aggregate GUD across sampling periods. For my analyses, I completed graphs across sampling dates for each experiment to allow for visual comparisons of the data. For each experiment, I then used a two-way ANOVA to analyze effects of Site and Habitat treatments on the average GUD (i.e., number of oil seeds remaining), where the data included in the analyses were averages across all four sampling periods for each bin, by treatment. Lastly, while not analyzed statistically, video observations of the different foragers were summed across sampling dates for all treatments and presented graphically.

III. RESULTS

When visually inspecting the graphs of seeds remaining (GUDs) across treatments, the only clear pattern is that the campus site generally had higher GUDs on individual sampling dates than did Jennings' Woods. While this trend was less clear in Experiment 1 (Figure 3), it was fairly strong in experiments 2 and 3 (Figures 4 and 5).

With respect to GUDS (averaged across sampling dates for each bin) in these three experiments, there was some variability relative to the effects of site and habitat on giving-up densities. In Experiment 1 (Figure 3), neither site (P = 0.11) nor habitat (P =0.78) significantly affected seed consumption (i.e., giving-up densities). In contrast, in experiments 2 (Figure 4) and 3 (Figure 5), the campus site had significantly higher numbers of remaining oil seeds (i.e., higher giving-up densities) than at the Jennings Woods site (Experiment 2: $P = 5.26 \times 10^{-6}$, Experiment 3: P = 0.035). However, habitat did not significantly affect giving-up densities in either Experiment 2 (P = 0.28) or Experiment 3 (P = 0.67). Further, no experiments had significant interaction effects (Site x Habitat; Experiment 1: P = 0.06, Experiment 2: P = 0.06, Experiment 3: 0.66).

When analyzing game camera video footage from Experiment 3, squirrels were only captured on video five times (at three total bins). One bin was in the campus open habitat, one was in the campus forest habitat, and the final bin was in the forest at Jennings' Woods. In all five instances, the squirrel was leaning in and feeding from the edge, and eventually, jumped inside the bin to feed. While they fed readily from the bins, four out of five squirrels maintained high levels of vigilance, with constant visual habitat assessments.

No chipmunks were captured on film, and the only other rodent that was observed was the white-footed mouse (*Peromyscus leucopus*), which was seen feeding in a forested bin and an open bin at Jennings' Woods. While few rodents were captured on film, there was plenty of footage of other animals feeding from the bins, particularly raccoons (which fed heavily from bins; Figure 6), some white-tailed deer (Figure 6), and various species of birds [e.g., black-capped chickadee (*Poecile atricapillus*), tufted titmouse (*Baelophus bicolor*), blue jay (*Cyanocitta cristata*), Northern cardinal (*Cardinalis cardinalis*), white-breasted nuthatch (*Sitta carolinensis*), and wild turkey (*Meleagris gallopavo*); Figure 7]. Opossums and Eastern cottontails (*Sylvilagus floridanus*) were occasionally seen, but tended to inspect the bin and move on without feeding (Figure 7), with only two instances where an individual sampled the seeds. From inspection of these graphs, it is clear that non-rodent foragers were much more active and abundant at the Jennings' Woods site than at the campus site.





Figure 3. Feeding Experiment #1 – 15 December - 23 December 2019. Top Panel: Treatment-specific average number of seeds remaining (\pm 1 SE) after a two-day period relative to sampling date (Sites: C = Campus, J = Jennings' Woods; Habitats: F = Forest, O = Open). Bottom Panel: Average number of seeds remaining (\pm 1 SE) across all sample dates relative to Site (Campus vs. Jennings' Woods) and Habitat (Forest vs. Open).



Figure 4. Feeding Experiment #2 - 2 January - 10 January 2020. Top Panel: Treatmentspecific average number of seeds remaining (\pm 1 SE) after a two-day period relative to sampling date (Sites: C = Campus, J = Jennings' Woods; Habitats: F = Forest, O = Open). Bottom Panel: Average number of seeds remaining (\pm 1 SE) across all sample dates relative to Site (Campus vs. Jennings' Woods) and Habitat (Forest vs. Open).



Figure 5. Feeding Experiment #3 - 7 March - 15 March 2020. Top Panel: Treatmentspecific average number of seeds remaining (\pm 1 SE) after a two-day period relative to sampling date (Sites: C = Campus, J = Jennings' Woods; Habitats: F = Forest, O = Open). Bottom Panel: Average number of seeds remaining (\pm 1 SE) across all sample dates relative to Site (Campus vs. Jennings' Woods) and Habitat (Forest vs. Open).



Figure 6. Non-rodent mammals observed in videos for Feeding Experiment #3 - 7 March - 15 March 2020. Top Panel: Treatment-specific sum of individual camera observations of raccoons at bins (summed across sampling dates), distinguished by whether or not they were feeding. Bottom Panel: Treatment-specific sum of individual camera observations of white-tailed deer at bins (summed across sampling dates), distinguished by whether or not they were feeding. Letters in treatment acronyms are as follows: Sites: J = Jennings' Woods, C = Campus; Habitats: F = Forest, O = Open)



Figure 7. Other animals observed in videos for Feeding Experiment #3 - 7 March - 15 March 2020. Treatment-specific sums of individual camera observations of opossums (blue bars) and birds (red bars) at bins (summed across sampling dates). Letters in treatment acronyms are as follows: Sites: J = Jennings' Woods, C = Campus; Habitats: F = Forest, O = Open

IV. DISCUSSION

Despite the outcome predicted in Hypothesis 1, there were no significant differences in foraging and giving-up densities associated with habitat, despite the expectation of differences in perceived risks associated with open and forested habitats. That said, while not statistically significant, forest habitat at the campus site had a higher giving-up density (more seeds remaining) than open habitat across all three experiments (unlike Jennings' Woods). Thus, while there were no significant effects of habitat on giving-up densities, there is a suggestion that further effort should be focused on exploring these relationships more deeply, given potential differences in predation risk between these two habitats. It might also be that the close proximity of the forest edge to the bins altered foraging decisions, as a result of the potential cover the forest edge provides, particularly for small, ground-foraging animals. Cover associated with the edge might have altered their level of routine vigilance. Also, it is worth noting that these experiments were done during winter, meaning that the trees had bare branches and may not provide as much protection as they would at other times of the year. This may account for some of the inconsistent data across the two habitats at these two sites.

Overall, there was a clear difference between study sites, with increased foraging activity in the less disturbed, forested site at Jennings' Woods relative to the more disturbed campus site with higher anthropogenic activity. In fact, for experiments 2 and 3, Hypothesis 2 was rejected given that the expectation was that there would be more foraging activity at the campus site than Jennings' Woods. This result was quite surprising given that previous studies found higher levels of foraging in more disturbed, urban sites, likely resulting from forager adaptation to human behavior (Bowers and Breland 1996, McCleery 2009). Adaptation to human activity is critical as it allows squirrels to spend time and energy focused on activities like foraging and reproducing, instead of constantly employing anti-predator behaviors in response to humans (McCleery 2009). These studies found a clear difference in the foraging behaviors used by animals that regularly feed in urban areas with higher anthropogenic activity, relative to animals that regularly feed in less disturbed rural areas (Bowers and Breland 1996, McCleery 2009).

The higher levels of foraging at Jennings Woods might be the result of site-to-site differences in the density of squirrels and chipmunks, as well as differences in the abundance and diversity of non-rodent foragers. If there are more animals (of all types) foraging at Jennings' Woods, it makes sense that there would be less food left in the bins than at the campus site. It is clear that this was an important factor in this study, especially considering the surprising game camera footage and lack of rodents. The difference between the levels of foraging at the different locations may be attributed to raccoons rather than squirrels and chipmunks. Raccoons were common foragers at Jennings' Woods but were less common at the campus site. Therefore, with a greater number of foragers, the site differences may be attributed to the entire community of foragers rather than the perceived predation risk by rodents. In addition, with a

potentially larger number of individuals in the larger foraging community, foraging activities and behaviors might well be altered.

Another explanation for this surprising result could be that while the campus location is in an area with more anthropogenic activity, there is not as much activity as in a city or park. This study site is an outdoor sports complex that is inactive in the middle of winter, so it did not see as much human activity as it might during other times of the year. Thus, squirrels and other foragers that might inhabit this campus location might show seasonal patterns of acclimation to humans and still avoid human presence and disturbance and be more vigilant when a human is nearby during winter. This type of seasonal change can be seen in other animals where vigilance is higher during hunting season, but lower during other times of the year (Jayakody et al. 2008). If this is the case, they would have to respond to both humans and their natural predators, whereas at Jennings' Woods, they would only have to respond to natural predators. This could account for higher giving-up densities at the campus site compared to Jennings' Woods.

Sampling Methods

Clear plastic bins proved to be an effective way of presenting a potential meal to mammals observed in this study. The degree to which they affected foraging depended upon the forager type and species. For example, the height of the bins was just enough that the squirrels and chipmunks would need to jump into them instead of just walking up and eating, meaning that they needed to work harder in order to forage inside the bins. In contrast, non-rodent foragers, such as deer and raccoons, could lean over the edge and

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feed directly from the bin. Further, the addition of plastic substrate to the seeds (in the third experiment) increased the difficulty of foraging, forcing all foragers to spend more energy sifting through the bins. Both aspects, the height of the bins and the plastic substrate, reduced the "free" aspect of the meal, without preventing foraging animals from feeding from the bins.

Effects of Predator Cues and Food Availability

Experiment #4 was originally designed to test Hypothesis 3 regarding effects of predator cues and food availability on habitat-specific foraging patterns. Unfortunately, due to the COVID-19 pandemic, this experiment was not completed. Although no data were collected, I would expect that the coyote urine would have deterred or reduced animal foraging in the bins it was sprayed around, leading to higher giving-up densities relative to the bins with no coyote urine.

The addition of coyote urine would likely have caused animals to switch between routine and induced vigilance. Squirrels would likely use routine vigilance while approaching the bin, but then switch to induced vigilance once they encountered the direct stimulus (coyote urine; Blanchard and Fritz 2007). This induced vigilance may cause the squirrel to freeze or run away, which routine vigilance may not cause on its own (Blanchard and Fritz 2007). If the animals chose to forage in enhanced predator cue bins, they would likely be more vigilant for the perceived predator, leading to reduced energy and time for collecting food, leaving more seeds left behind. This expected result is supported by the fact that predator urine has repelled prey foraging in previous studies. For example, the presence of urine from coyotes (*Canis latrans*) and bobcats (*Lynx rufus*) can reduce browsing on Japanese yews (*Taxus cuspidate*) by white-tailed deer (*Odocoileus virginianus*) (Swihart et al. 1991).

With respect to food availability, I would have expected increased foraging time (and lower GUDs) on the high food availability treatment (relative to limited food availability) as there would have been a significant reward associated with foraging. Ultimately though, the coyote urine would likely have reduced this difference between food availability treatments due to an increase in induced vigilance.

It is difficult to tell whether habitat-specific giving-up densities would have been affected differently by coyote urine. One might predict that the predator cue would overwhelm effects of habitat on giving-up densities. However, in one study, fox squirrels responded more strongly to feeding site than to olfactory cues from a predator (Thorson et al. 1998). Given this, it is difficult to predict the relative importance of habitat and predator urine to determining giving-up densities.

<u>Potential Sources of Error in this Study</u>

When conducting these types of experiments, it is important to consider aspects of the study design that might have affected the outcome of the experiments. One potential issue in this study was the weight of the bins. Occasionally, bins were found flipped over or in a different location than where they were originally placed, even with the large rock in each bin. If seeds spilled onto the ground when the bins were flipped, it would have changed the foraging environment, possibly affecting the level of perceived danger or safety (along with possibly affecting seed counts). Similarly, if a bin was moved closer to the tree-line (Note: this edge could be characterized as an ecotone) from the open habitat, this also could have altered the perceived foraging risk, in turn affecting the giving-up density and number of seeds remaining.

Another potential issue in this study is that there was no way to control which animals fed from the bins. While the study focus was primarily on the rodents, it appears that there was a lot of non-rodent foraging in the bins by deer, raccoons, opossums, and different bird species (e.g., black-capped chickadee, tufted titmouse, blue jay, Northern cardinal, white-breasted nuthatch, and wild turkey). The presence of these other foragers may have affected feeding patterns by squirrels and chipmunks, both through interference and disturbance, as well as altering food availability and their subsequent foraging decisions. While not unexpected, given that raccoons (*Procyon lotor*) and opossums (*Didelphis virginiana*) foraged in the bins at nighttime in the trial run of this study, the low number of documented foraging events by rodents was surprising.

Future Studies

If this study was repeated, there are many different ways we could alter the experimental design to get better insight into habitat or site effects on giving-up densities. Additional habitats that may differ in risk could be added or we could look at a range of distances from habitat boundaries to get a better sense of habitat effects. For example, one could place multiple bins in the open and forested habitats, at different distances from the tree-line. In this study, all bins were 3 m from the forest edge, but imagine if

you also placed bins 5, 10, 20, 40 m or more from the edge (where possible). The results could be similar to a study done with Vervet monkeys (*Chlorocebus aethiops*) which found that as distance from trees (a perceived safe habitat) increased, feeding intensity decreased (Makin et al. 2012). A design like this could give you a very different picture of habitat effects on giving-up densities, particularly in the open habitat, where as you got farther from cover, one would expect the perceived risk to be higher.

Another interesting change to this design would be to add new habitats. For example, adding a new habitat such as an arboreal feeding site might show differences in giving-up density relative to ground feeding sites (Makin et al. 2012). Squirrels are very good at navigating trees, so it would be interesting to see if they feel safer there or perceive any predators in the trees that would alter their giving-up density. Previous research has documented that as habitat complexity increased (e.g., by adding a vertical dimension), animal feeding preferences also became more complex (Laundré et al. 2010, Makin et al. 2012, Mella et al. 2014).

With respect to using different sites, locations with higher levels of human disturbance may give more insight into whether the human presence was a factor in the significant differences between the campus and Jennings' Woods sites. While it is oncampus, the Allerton Sports Complex is not as frequented by students as other parts of the campus. A potential third experimental site could be more central campus, or perhaps John Brown Tannery Park, located in downtown Kent, Ohio. This park is frequented by many walkers and joggers, and would provide a greater human presence than Allerton Sports Complex. This may provide more insight as to how much human presence affects foraging activity in squirrels. Therefore, increasing both the spatial and temporal scale of the experiment may give further insight into how human behavior affects foraging activity; both through an increased duration of the experiment to incorporate seasonal variability and a spatial increase of adding a new location could show differences in foraging activity and behavior (Levin 1992). However, this may be difficult because walkers on the trail may be compelled to pick up the bins if they think they are litter.

Lastly, one aspect that could be improved upon, particularly when thinking about the reward side of the risk-reward balance these animals need to account for, is food availability in the bins. One could alter the number of seeds placed in the bin, and/or how frequently they are replaced. For this experiment, 500 seeds were placed in each bin. This was because counting seeds took a considerable amount of time and this was the most reasonable number of seeds that could be counted in-between emptying the bins and replacing seeds. However, there may not have been enough seeds to see a strong difference in giving-up densities. It is possible that the squirrels, and other animals, could have eaten through the 500 seeds too quickly to see significant treatment differences among the bins. Instead, it might be beneficial to put more seeds in the bins, so that the animals would not be able to consume them all between seed replacements. One option would be to put a set weight of seeds into bins to avoid the time-consuming counting process, with the weight of seeds remaining being determined after replacement. In fact, we took this approach for Experiment #4 with the two weight-based food availability treatments. However, because we ended it, we were not able to determine if this approach made a difference. In addition, a future study could replace the seeds every day, instead

of every other day as we did, which would reduce the likelihood that all of the seeds would be eaten, and we might get a better estimate of giving-up density.

Implications of this Study

In the end, investigating the foraging activity and giving-up densities of squirrels and chipmunks proved to be complex. It was especially challenging given the fact that it was impossible to exclude other animals from feeding from the bins, which may have affected food availability and altered the feeding behavior and activity of squirrels and chipmunks. However, clear differences between the sites gave some insight on how animals forage in these settings. While we did not learn much about factors influencing rodent foraging, we did gain insight into the complex and diverse array of animals foraging at these sites, with the suggestion that competition or interference may affect these relationships as well. Site differences in animal behavior and giving-up densities also suggest that animals are adapting to human presence even at locations we perceive to be less disturbed. Thus, these types of studies can provide insight as to how animal behavior may change with the ever-increasing urbanization of the planet

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