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The Effects of Auditory Predator Cues on Foraging Behavior in
Peromyscus polionotus

An Honors Thesis submitted in fulfillment of the requirements for Honors in Biology.

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Abstract

While it is established that animals react to predator cues, little research has been done to assess its effect on foraging patterns and behavior. Foraging patterns are determined by a variety of factors associated with perceived predation risk, such as vegetation cover and moonlight exposure. Predator cues come in many forms, both direct and indirect, and are another indicator of perceived predation risk. Past research suggests that oldfield mice, *Peromyscus polionotus*, may react to indirect cues (i.e., vegetation cover) more often than some direct cues, such as urine of a predator. I predicted that hearing a known predator (i.e., direct cue) call near a burrow during active hours would significantly increase the GUD, a measure of perceived predation risk. I conducted this experiment between the winters of 2018-2019 and 2019-2020. Responses to direct cues were measured, while indirect cues, such as vegetation cover and moonlight were minimized. The results were mixed. During the first winter, the GUDs were significantly higher in response to predator calls. In the second winter, GUDs were similar across treatments. These results suggest that auditory cues may influence foraging behavior in oldfield mice (*P. polionotus*), but more research needs to be done to determine if the effect is dependent on other variables such as weather.

Background and Significance

The ability to assess predation risk increases an animal's chance of survival and enhances fitness. Predator cues, or signals from a predator that the prey receive, help the prey animal to identify the predator and attempt to avoid predation. Both direct (visual, olfactory, and auditory) and indirect cues (injured conspecific, increased exposure, etc.) are used by animals to assess predation risk (2,3,11). Numerous studies have evaluated the importance of these cues in prey behavior (3,4,6,7,8). For example, Mirza et al. exposed tadpoles to a chemical predator cue, the odor of dragonfly larvae, and recorded their response. Large amounts of odor (1:1) elicited an anti-predator stress response, while small amounts (1:1000) did not (7). Thus, in some species it is clear there is a reaction to predator cues, which could affect fitness levels. During foraging, animals are physically exposed and at risk of predation. If certain individuals excel at avoiding predation, and the characteristics that allow them to avoid predation by recognizing predator cues are genetic, they will pass those traits on to their offspring. In response, predators co-evolve to keep up with predation avoidance in prey, resulting in an “arms race” over time. Thus, predatory cues become harder and harder to detect, and prey continually improve their detection of cues. The importance of understanding foraging behavior has diverse importance. When behavior and feeding patterns are mapped out for any species, the relationships of coexistence within an ecological community are made clearer (15), and the habitat is better understood.

Animals must balance the risk of predation with their survival and reproductive needs. Foraging behaviors are determined by a variety of factors and are strongly influenced by perceived predation risk. Perceived predation risk includes the presence of predator cues.

Predator cues come in these forms: olfactory (urine, musk, pheromones etc.), visual (observing a predator, or observing an injured conspecific), and auditory (hearing a predator cue). In kangaroo rats, body size and gait effect foraging preference, but the presence of a predator shifted kangaroo rat foraging patterns. When predators were at a high density, *Microdipodops megacephalus* foraged at smaller distances and ate clumped seeds more often than when predators were at a lower density, suggesting that predator density, along with other factors, influences foraging behavior. (14). Many studies have evaluated visual and olfactory (13,14) cues, but relatively few have evaluated auditory cues (14), and those that have shown conflicting results. In one study, Webster and Webster (1971) found that kangaroo rats were more often struck by sidewinder rattlesnakes when middle ear volume was surgically reduced than when not (14), suggesting that auditory cues may be used in predator avoidance. However, when Millus and Stapp studied the relationship between seabirds calls and endemic deer mice, they found that habitat factors affect population numbers more than the seabird population (6). In rodents in particular, research of response to predator cues has focused on visual and olfactory cues (8,7,5). Several studies of squirrels and mice have demonstrated the effects of perceived predation risk, altering when, where, and how much foraging is done in response to predator cues (2,3,5). However, the use of auditory cues in mice for any purpose is only beginning to be explored.

Oldfield mice are small monogamous mammals common in early successional habitats in the southeastern U.S move that habitats frequently and interact with their community in vital ways. Much like the Gopher Tortoise, the burrows dug by *P. polionotus* (Figure 1) provide unique shelter for many other organisms in their environment, protecting them from scheduled burns, weather, and predators. Additionally, this work is important for conservation. Several

subspecies of this mouse are endangered (2). Thus, understanding the drivers of foraging success is important.

The purpose of this study is to test whether direct predator cues in the form of predator calls effects foraging effort in *P. polionotus* as measured by giving up density (GUD). In previous studies, *P. polionotus* were more likely to forage under vegetation cover (5,8), where open space is an indirect predator cue. In both studies, GUD was higher in trays farther from the nesting burrow, an indirect cue of higher perceived risk. These same studies found that mice were less responsive to direct predator cues, such as the smell of predator urine, than they were to indirect cues. Other studies have also found that oldfield mice respond to direct predator cues more weakly than indirect cues (12). In this study, indirect cues will be minimized to test for the effect of a direct predator cue (sound). To the best of my knowledge, response to predator calls has not been tested in this species. My hypothesis is that direct predator cues, in the form of auditory calls, will significantly affect foraging effort as measured by the giving up density (GUD). I predict that *P. polionotus* will demonstrate a higher GUD in experimental trays emitting Barn Owl (*Tyto alba*) and coyote (*Canis latrans*) calls when compared to white noise (cricket calls) or no noise. Both of these predators are found locally, with coyotes being more common than barn owls. Both are nocturnal predators in oldfield mouse habitat and are active when *P. polionotus* forages.



Figure 1. *Peromyscus polionotus*

Methods:

I located active *P. polionotus* burrows in an open field in Candler County, Ga. at 32°26'47.6"N 82°03'15.2"W in the winter of 2018-2019 and 2019-2020 (Figure 2, 3). No other rodents in this area burrow; old-field mouse burrows are characterized by an apron and are distinctive (1). Oldfield mice are an early successional species that prefer old-field habitats with fairly homogeneous vegetation. After locating burrows, I marked active burrows and selected 4 to use in the experiment. Burrows used in the experiment were at least 25 m distant from one another. At each burrow, I placed three 22.6 oz plastic containers (serving as a foraging tray) and an iPod emitting a recording of a predator call or other sound. The 3 trays served as replicates for one treatment (one treatment at a time per burrow). The foraging trays each contained 3.1 grams of millet and 200 ml of clean sand, along with two equally sized holes on either side for access by mice. The trays were closed with a lid to prevent disturbance by other species, such as birds and larger species of rodents or other mammals. The holes were located on the short ends of the containers and are approximately 1 in 2, a size small enough that only tiny mice can access. The

iPod and portable battery were also enclosed in a plastic container to maintain a dry environment; this container was perforated with small holes so that sound could be easily heard (Figure 4,5). At each burrow, a different recording was played (barn owl, coyote, white noise, no noise). Calls played intermittently on a loop for a period of 2 minutes every 4 hours during nighttime hours (approximately 12 hours). The white noise and no noise sites served as controls. At the end of each night, the seeds remaining in the trays were collected, the trays were refreshed, and treatments were rotated. Treatments rotated among mouse burrows nightly; data were collected for 3-4 nights. For example, if on night 1 treatments were 1-coyote, 2-barn owl, 3-cricket, 4-silence, then the following night treatments were 1-silence, 2-coyote, 3-barn owl, 4-cricket. At the end of the experimental period, the trays were refreshed and left undisturbed for one week. The following week the trials were repeated (weather permitting). To minimize indirect cues, moonlight exposure and vegetation cover were avoided. Small mammal activity is generally greater during a new moon and less during the bright light of a full moon, although there is variability among species (3,4,5,9). This experiment was conducted during two winters, taking place in the months of October 2018-March 2019, and then again January-March 2020. After the replications were completed, data was examined to determine if hearing a predator altered foraging activity based on the giving up density (GUD), which is determined by the weight of seeds left in the tray (8,9). Data were analyzed using JMP Pro 13. Treatments were compared using a one-way analysis of variance (ANOVA); each year was analyzed separately because there was a significant effect of year. All pairwise comparisons were tested using Tukey-Kramer tests. The experimental protocol was approved by the Georgia Southern IACUC committee IACUC #19004.



Figure 2. The map and locations of active burrows used in trial 1 of the experiment. Each active burrow site is indicated with an "X". Trial 2 took place in the same area, with varying burrow locations.



Figure 3. Typical oldfield mouse habitat in Candler County, GA.



Figure 4. An example of an active burrow site, complete with three foraging trays and the iPod container

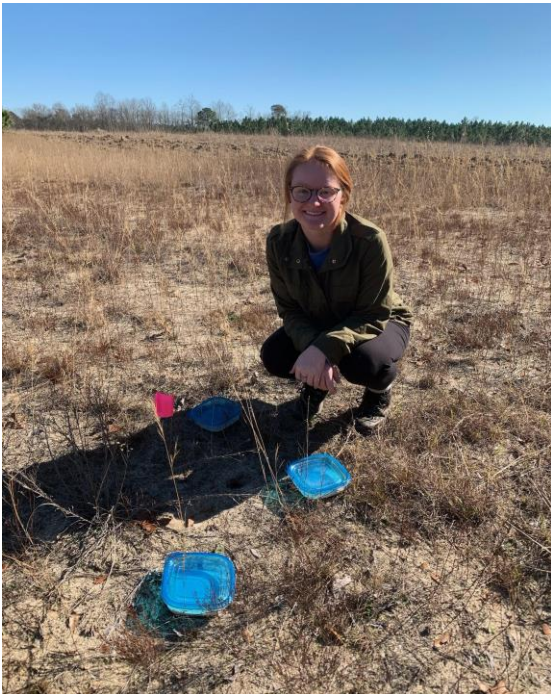


Figure 5. Christina with an active burrow site for scale.

Results

Year 1

There were 17 experimental nights in year 1. There were 4 treatments; each treatment had 45 replicates except crickets, which had 42. There was a significant treatment effect ($F=4.69$, d.f. = 3, $p < 0.0036$). All GUDs for barn owl, coyote, crickets, and silence were between 0.7g and 1.3g. Coyote call treatments had higher GUD than both silence ($p < 0.0193$) and cricket ($p < 0.0225$; Figure 6). GUDs in all other pairwise comparisons were similar (Figure 6).

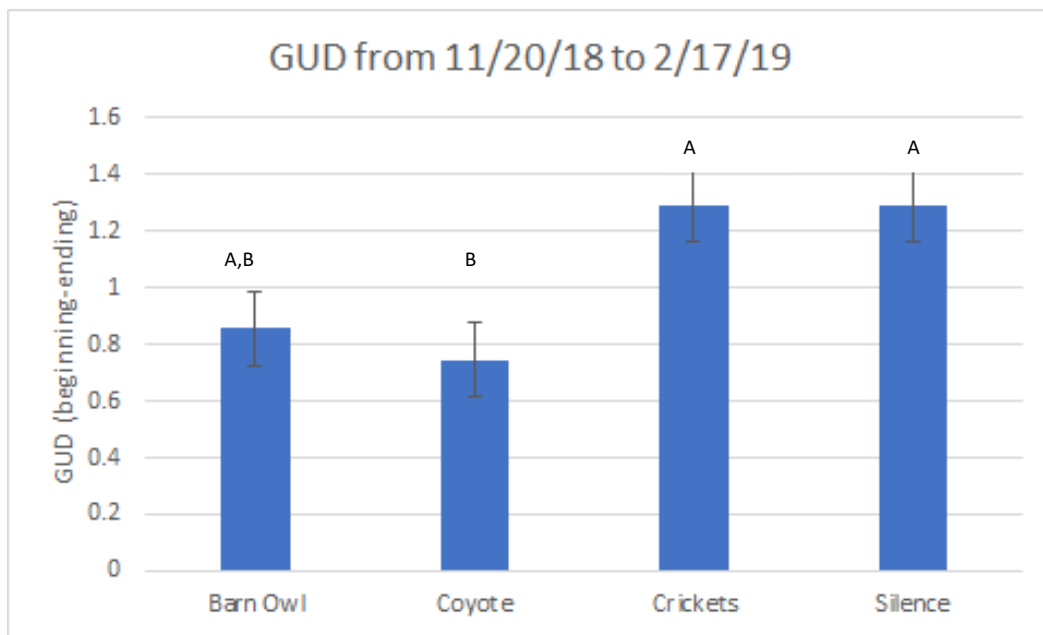


Figure 6. GUD measured from 11/20/18 to 2/17/19. Difference in the weight of seeds remaining in a foraging tray after a 24-hour trial period where a predator call was played (means \pm SE). Columns with no letters in common are significantly different ($p < 0.003$).

Year 2

There were 10 sample nights in year 2. The same four treatments were used as in year one.

Silence, coyote, and crickets had 30 replicates. Barn owl had 29. There was not a significant treatment effect ($F=0.7959$, d.f. = 3, $p > 0.4986$). All GUDs for barn owl, coyote, crickets, and silence were between 1.0-1.50 (Figure 7).

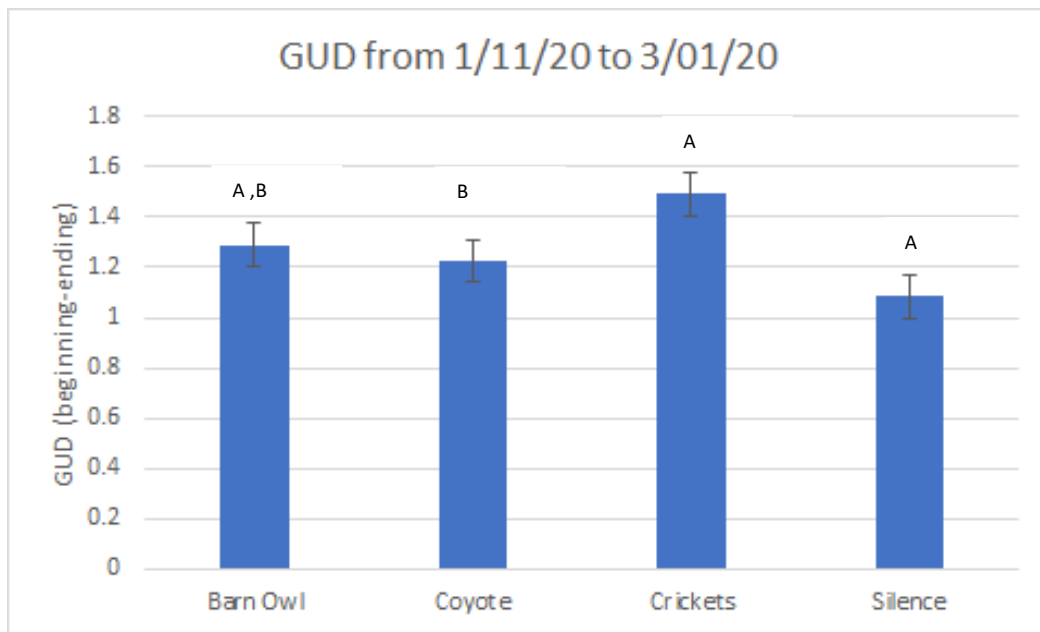


Figure 7. GUD measured from 1/11/20 to 3/01/20. Difference in the weight of seeds remaining in a foraging tray after a 24-hour trial period where a predator call was played (means \pm SE). Columns are not significantly different ($p > 0.4986$).

Discussion

My prediction that the GUD would be higher in experimental trays with predator calls

than at trays assigned white noise/control was supported in year 1 (Figure 6). This suggests that auditory predator cues do affect foraging behavior, but that mice do not discern between specific predator calls. This result is different than previous findings, which suggested that indirect cues, rather than direct cues like predator calls, affect foraging behavior (6). However, data from year two did not support my prediction; there was no significant difference between experimental and non-experimental trays. The explanation for the difference between years is unclear, and suggests that other variables, such as weather, may play a role in determining whether or not direct cues influence foraging behavior. Some notable differences between the two trials are worth mentioning. During year one, trials were conducted over a 3.5-month period, with longer breaks in between experimental conditions. During year two, trials occurred more consecutively during the late winter/early spring over a period of 1.5 months. There was considerably more precipitation during year 2, with an average rainfall amount of 9.295 inches per month. Trial one's period saw an average rainfall amount of 8.44 inches per month (13). It was also considerably warmer in trial two than in trial one. Additionally, more individual burrows were used in year two (and thus more individual mice), perhaps leading to higher between individual variation. Finally, because four burrows were repeatedly sampled in year one versus eight in year two, the area sampled in year one was smaller. Previous studies, including Orrock et al (9,10,11), studied mouse foraging across one season, and found that indirect, rather than direct predator cues affect foraging patterns. These studies all took place in the summer months and did not observe foraging behavior in the winter seasons, whereas this study took place over two years in the winter months. It is also possible that *P. polionotus* can learn to use indirect predator cues as a warning, and that it is not an innate behavior (14). If it is indeed a learned behavior, mice in certain areas would avoid predators common to that area, while others do not. It could also be

seasonal. In the winter, there is less greenery to shade mice from avian and mammalian predators, and so indirect cues could prove more effective in the winter than in summer months where there is sufficient vegetation cover (5). This study minimized the effects of indirect cues to look for an effect of direct cues with mixed results.

Future studies should examine the effects of individual variation and inter-annual variation, as these may impact foraging behaviors. Ideally, a field project of this nature should be conducted over the course of several consecutive seasons, statistically correcting for any differences in precipitation and temperature and standardizing other indirect cues, such as vegetation cover. It should also include a biological survey of the area, with confirmed and common sightings of all predators used. It is also possible that individual *P. polionotus* can learn to use indirect or direct predator cues, and that it is not an innate behavior (14). If it were indeed a learned behavior, mice in certain areas would avoid predators common to that area, while others would not. Therefore, in addition to a more detailed field experiment, a lab experiment detailing mouse behavior when presented with a predator sound and no other cue would provide insight into the behavior of different individuals. This would help discern innate from learned behavior and could solidify supporting data.

My experiments and research have proved inconclusive on whether or not *P. polionotus* take auditory predator cues into account when foraging, but further research and more meticulous methods could potentially find that they do. These creatures are the base of a healthy food chain in our temperate forest and understanding how and why they feed could one day help us grasp the complexity of the balance of our ecosystem.

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