

The Log Less Travelled:

How different temporal conditions effect the pathway selection of Woodland Deer Mice,

Peromyscus maniculatus gracilis

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Margrethe L. Andreasen

Advisor: Michael Cramer

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ABSTRACT

For prey animals such as the woodland deer mouse, *Peromyscus maniculatus gracilis*, behavior reducing their risk of predation is vital to their survival and influences almost every aspect of their life, including route selection. This study evaluated the routes of 16 adult deer mice in two different temporal settings (night and day) to assess different anti-predator strategies. Contradictory to popular literature, the mice were not found to prefer quieter substrate overall, and there was no noted difference in substrate preference (loud leaf litter vs. quiet coarse woody debris) between the two trials. Anti-visual detection behavior was observed in the significant difference of distance traveled between day ($12.040 \text{ m} \pm 3.576$) and night ($32.375 \text{ m} \pm 7.450$). More intensive behavioral research is necessary to fully understand the influence of predation reducing behavior on the pathway selection of deer mice.

KEYWORDS

Peromyscus maniculatus, route selection, pathway selection, microhabitat selection, decision making, anti-predator behavior, leaf litter, coarse woody debris, moonlight illumination

INTRODUCTION

Due to the effect of death on an animal's fitness, much of an animal's morphological traits and modern behaviors exist to decrease the risk of predation (Lima and Dill 1989). Some behaviors that act to decrease the risk of predation are subtler and can be observed in the animal's decision making; a dynamic example of this is route selection. This form of decision making is influenced by multiple environmental conditions such as illumination (Barry and Francq 1982, Travers et al. 1988, Daly et al. 1992), substrate type (Roche et al. 1999, Hinkelman et al. 2011), vegetation cover (Travers et al. 1988, Barnum et al. 1992, Hinkelman et al. 2011), and greatly depends upon the type of predation risk. Little can be done in modern timescales to decrease the risk of olfactory detection, but auditory and visual detection can, and often is, taken into account in prey decisions

In the case of auditory detection and route selection, quieter paths such as those with more coarse woody debris or damp leaves are more heavily selected (Fitzgerald and Wolff 1988, Barnum et al. 1991, Roche et al. 1999). These substrate preferences have been well studied within the deer mouse, *Peromyscus maniculatus*, and other small mammals. In an enclosure experiment, Roche et al. (1999) determined that deer mice, *Peromyscus maniculatus*, prefer to travel on fallen logs and other coarse woody debris (CWD) rather than the louder leaf litter, but when forced to travel on leaf litter, the quieter coniferous needles were more heavily selected for. A study by Barnum et al. (1992) observed similar results in *P. leucopus* individuals, where the quieter smooth or moss-covered logs were preferred. It was also found that within leaf litter preferences, dampened leaves were more heavily preferred by *Peromyscus spp.* due to their quieter nature (Fitzgerald and Wolff 1988, Roche et al. 1999).

Behaviors decreasing the risk of visual predation is well documented within *Peromyscus spp.* and *Peromyscus maniculatus* in the form of cover and illumination preference (Travers et al. 1988, Brillhart and Kaufman 1991, Barnum et al. 1992, Jacob et al. 2017). Deer mice already diminish visual predation risk by reducing daytime activities, and have been shown to further decrease their activity on more illuminated nights (Barry and Francq 1982, Bowers 1988, Brillhart and Kaufman 1991, Daly et al. 1992). On brighter nights deer mouse and small mammal use of vegetation cover also increases, a further step to reduce visual predation risk (Clarke 1983, Travers et al. 1988, Barnum et al. 1992, Jacob et al. 2017). This goes not without valid reason; the predation and successful capture of deer mice by short-eared owls (*Asio flammeus*) increases on more illuminated nights (Clarke 1983). Although the visual acuity of deer mice is more heightened than that of most rodents (King and Vestal 1974), and thus moonlight can be used to their advantage (foraging, mating, etc.), the minimization of predation risk is their top priority and most greatly influences their behavior.

In order to further investigate how deer mouse path selection behavior is affected by the risk of predation, I tracked the routes of released woodland deer mice, *Peromyscus maniculatus gracilis*, during peak daylight and night hours in the Northwoods of Wisconsin. The routes were assessed in terms of length, direction, general coverage, general illumination level, and substrate. Two hypotheses were tested: 1. night trial mice should show a stronger preference for quieter substrates compared to day trial mice, and 2. there should be a stronger preference for coverage during the day trials compared to the night trials.

MATERIALS AND METHODS

Study Sites

Peromyscus maniculatus gracilis is found throughout the forests and fields of the University of Notre Dame Environmental Research Center (UNDERC), located on the border of the Upper Peninsula of Michigan and northern Wisconsin (46° 13' N, 89° 32' W). UNDERC is an area of about 6153 acres with 30 lakes and bogs with a combined area of 1347 acres and mostly northern mesic forests (Curtis 1959). The sites for trapping and release consisted of mixed hardwood forests (*Acer saccharum*, *Acer rubrum*, *Abies balsamea*, *Picea glauca*, *Betula alleghaniensis*, *Tsuga canadensis*), with little to no understory growth. Six sites were chosen for this specific forest composition and predicted high *Peromyscus spp.* density (Figure 1).

Trapping Protocol

P. m. gracilis individuals were caught via live-trapping in July 2019. Trapping grids contained 25 traps in a 5 x 5 configuration with 15 m spacing, or a half grid containing 15 traps in a 3 x 5 configuration, 15 m spacing. All mice were live-trapped using Sherman traps (7.62 x 8.99 x 22.86 cm; H. B. Sherman Traps, Inc., Tallahassee, FL) baited with sunflower seeds. When captured, mice will be identified as *P. m. gracilis* using ear length (Lindquist et al. 2003; Ridenhour and Cramer 2015), and then sexed, weighed, and marked with ear tags (monel 1; National Band and Tag Co., Newport, KY).

Field Site Description

The grids and half grids were sectioned into quadrants (Figure 2). The center point of each of quadrant was used as a common release point for any mouse caught within said quadrant. For the mice on the gridlines between two or more quadrants, the quadrant of their release was determined randomly.

Experimental Procedure

Following capture all adult mice, excluding lactating and pregnant females, were taken to the laboratory and housed in individual cages (19 x 29 x 12.5 cm) for a minimum of 30 hours and a maximum 40 hours. The captured adult mice were given a mix of paper fiber and corn grains for bedding, polyfill nesting material, and water and food (rat pellets, sunflower seeds) *ad libitum*.

For the trials, mice were randomly selected to be released during the day between 1100 and 1400 or at night from 2100 to 2300. The mice were transferred into Sherman traps and transported back into the field, where they were again transferred to a “rainstick”. The rainsticks are approximately 30 cm long 1 ¼” polyvinyl chloride (PVC) pipe with capped ends. The three rainsticks each contained approximately a teaspoon of BioQuip Luminous Powder (BioQuip Products, Inc., Rancho Dominguez, CA) in either yellow, red, or blue. Different rainsticks were used for each powder to avoid cross-contamination of colour, and different colours were used for multiple releases within the same area. Once the mouse was inside the capped rainstick, the rainstick was slowly inverted multiple times in a span of 30 s to 1 minute to ensure total coverage of the mouse by the powder.

After powdering, the mouse was released near the quadrant’s center flag in an arbitrary direction. Upon release, myself or my assistant would videotape the mouse using an iPhone XR (Apple Inc., Cupertino, CA) within the best of our ability without following the mouse closely, to avoid further distressing the mouse or affecting the mouse’s path. A timer was set upon release as well, and after 10 minutes data collection would begin.

Data collection begun by following the mouse’s trail of fluorescent powder using a portable ultraviolet flashlight (Glossday). Stake flags were placed at turns and substrate changes in the path. The path was followed to the point the mouse was known to have taken until the 10-

minute mark, or to the greatest extent possible given the visibility of the powder. Following the day trials, distance, direction, canopy density, herbaceous layer coverage (existence of plant coverage shorter than 50 cm), and substrate type was recorded. The night trial data were recorded the following day for simplicity.

Statistical Analysis

Proportion of total distance traveled on leaf litter (hardwood forest leaves) versus CWD (logs, tree roots, stumps, bark), total distance, total number of path deviations, and proportion of path distance spent exposed (not under herbaceous coverage) was analyzed for each trial type (night and day). All statistical analyses were conducted with R Studio Version 1.1.463 (R Development Core Team 2008). All animal use complied with the American Society of Mammalogists guidelines (Sikes et al. 2011). All statistics are reported as means \pm standard error of the means.

RESULTS

A total of 16 adult mice (12 male, 4 female *Peromyscus maniculatus gracilis*) were evaluated for route selection. More males were used in trials due to the presence of pregnant or lactating females. Total distance was not normally distributed (Shapiro-Wilk test: $W = 0.864$, $P = 0.022$), nor was the variance homogenous ($\chi^2 = 84.738$, $df = 1$, $P = 0.000$), so a Kruskal-Wallis test rather than a one-way ANOVA was done and revealed night trial mice paths ($32.375 \text{ m} \pm 7.450$) were significantly longer than day trial mice paths ($12.040 \text{ m} \pm 3.576$; $\chi^2 = 4.4118$, $df = 1$, $P = 0.036$). The deviation count between night and day trials was also not normally distributed (Shapiro-Wilk test: $W = 0.878$, $P = 0.036$), nor was the variance homogeneous ($\chi^2 = 77.968$, $df = 1$, $P = 0.000$), but the night mice deviation count (34.000 ± 5.435) was found to be significantly

different from day mice deviation count (15.750 ± 2.827 ; $\chi^2 = 6.372$, $df = 1$, $P = 0.0116$) using a Kruskal-Wallis test. A proportion of total distance spent outside of cover, referred to as distance exposed, was not normally distributed as well (Shapiro-Wilk test: $W = 0.871$, $P = 0.029$) nor had homogeneous variance ($\chi^2 = 13.254$, $df = 1$, $P = 0.000$), but was not significantly different between night (0.749 ± 0.075) and day trials (0.743 ± 0.116 ; $\chi^2 = 1.337$, $df = 1$, $P = 0.248$).

The proportion data of distance spent on different substrate type (leaf litter or CWD) were normally distributed (Shapiro-Wilk test: $W = 0.897$, $P = 0.073$). The data were then evaluated using a one-way ANOVA, with no significant difference found between the night (0.232 ± 0.082) and day proportions of time spent on CWD compared to leaf litter (0.279 ± 0.070 ; $F_{1, 14} = 0.189$, $P = 0.670$).

DISCUSSION

Although a strong preference for a substrate type was shown in both day and night trials, the stronger preference for leaf litter to coarse woody debris (CWD) was unprecedented and nonsignificant between the different temporal trials. This contradicts the first hypothesis predicting a stronger preference to the quiet substrate (CWD) in the night trials to the day trials, and previous studies that displayed a preference for CWD to the noisier leaf litter, regardless of light level (Roche et al. 1999). This contradiction implies that the deer mice are not as concerned about the risk of auditory detection as previously thought; the rustling of leaves produce sounds (8 kHz; Payne 1971) well within the audial range of many small mammal predators (Roche et al. 1999). A similarly surprising result was found by Hinkelman et al. (2011) involving decreased foraging around and on CWD. Much like my own study and Roche et al. (1999), Hinkelman et al. (2011) expected CWD to be used more often than leaf litter in foraging behavior. After

discovering that this was not the case, it was concluded the lack of CWD preference was most likely due to the use of the logs by snakes (primarily Viperidae) as ambush sites (Reinert et al. 1984). However, in the northern hardwood forest where this study took place, only snakes of the Colubridae family are found, and none are classified as potential predators for deer mice (Sullivan 1995). Thus, decreased use of CWD due to potential threats is an unlikely conclusion for this unexpected preference.

A possible explanation for the strong preference towards leaf litter could come from the weather conditions. General moisture conditions were recorded for each trial (wet vs. dry), and no more than 1.27 cm of precipitation was recorded for the days wherein trials were completed. No trials were completed during rain. Of the 16 trials, 8 were completed in dry conditions and 8 in wet. Five of the wet conditions were night trials, 3 day; three of the dry conditions were night trials, 5 day. The proportions of distance spent on leaf litter and CWD were evaluated for normality using a Shapiro Wilk test ($W = 0.897$, $P = 0.072$), and preference between condition type (wet vs. dry) using a one-way ANOVA. There was no significant difference in proportion of distance spent on CWD compared to leaf litter between the wet trials (0.299 ± 0.066) and dry trials (0.212 ± 0.079 ; $F_{1, 14} = 0.696$, $P = 0.418$). This lack of preference between dry and wet leaf litter further contradicts the experimental results of Roche et al. (1999) in *P. maniculatus*, the results of Fitzgerald and Wolff (1988) in *P. leucopus*, and my first hypothesis. The deer mice appear to display no behavior that reduces the risk of aural detection by predators.

Between the day and night trials there was a lack of significant difference in cover preference (no cover or cover), rejecting my second hypothesis. The simplest explanation for this behavior is, regardless of time, deer mice exhibit similar anti-predator behavior, likely caused by the ever-present risk of visual predators and predation (Sullivan 1995). This explanation is

supported by the preference for cover than no cover. An abiotic factor unaccounted for in the study could also be the cause of this lack of preference, the moon's illumination. For the week of trial releases (July 12th to July 17th, 2019), the moon transitioned from waxing gibbous to waning gibbous (Calendar n.d.) and was at peak illumination. A study done by Jacob et al. (2017) showed that foraging decreased in exposed forest areas (lacking vegetation cover) during times of increased illumination in *Peromyscus spp.* Thus, this constant high level of illumination present during the night trials may have increased herbaceous cover use, skewing the results in an unanticipated way. Further studies investigating moonlight effects on *P. maniculatus* vegetation cover use is necessary to understand these results.

The shorter distance traveled by day mice and lack of climbing observed in day mice is the best evidence for predation risk decreasing behaviors in deer mice (*Figure 3*). Excluding one day trial, all day mice returned to a relatively secure, hidden location (tree stump, hollow tree, branch pile) that was nearby, whereas many of the night trial mice (4; trials 8, 11, 13, 17) had no secure end location and traveled far enough that the fluorescent trail was unable to be accurately tracked. Furthermore, no day mice climbed a tree higher than the 1 m mark, while 3 of the night mice (trials 5, 10, 16) climbed and remained in trees. These results display the priorities of the mice, and help to further explain my other, contradictory results; the day mice did not display other behaviors decreasing visual detection (no cover preference) because they are more concerned with finding a secure location in less distance. Additionally, a behavior known as intermittent locomotion was informally observed by myself and fellow researchers only in day trial mice. Intermittent locomotion characterizes prey animals and helps avoid visual detection by predators (Kramer and McLaughlin 2001), thus the day trial mice are displaying some behaviors to minimize predation risk, just not the predicted ones. Further research is needed to

understand the priorities of deer mice when it comes to reducing predation risk and reaching a secure location.

The limitations of this study are evident in the low sample size and lack of constant environment. Because familiarity is shown to effect small mammal route selection (Clarke et al. 1993, Stamps 1995), the mice were released in the same grid quadrant as they were caught. This caused changes in environment that may have accounted for substrate choice and vegetation cover. Further studies should be done in the same area or should create a scale to adequately compare vegetation cover and substrate type of different areas. Additionally, the prevalence of lactating and pregnant females in the research area caused for a biased for male mice. Future studies should focus on only one sex, or investigate the possible difference between male and female deer mice locomotion and route selection.

However, this study shows important contradictions in previously accepted anti-predator behavior of deer mice. The lack of preference for coarse woody debris compared to hardwood leaf litter across is reason to believe deer mice actively make no behavioral changes to avoid auditory detection, and more field research is needed to further understand the substrate preferences in route selection. This study does support evidence for behavioral changes in regard to visual detection by predators as seen in the significant difference in distance traveled between the night and day trials.

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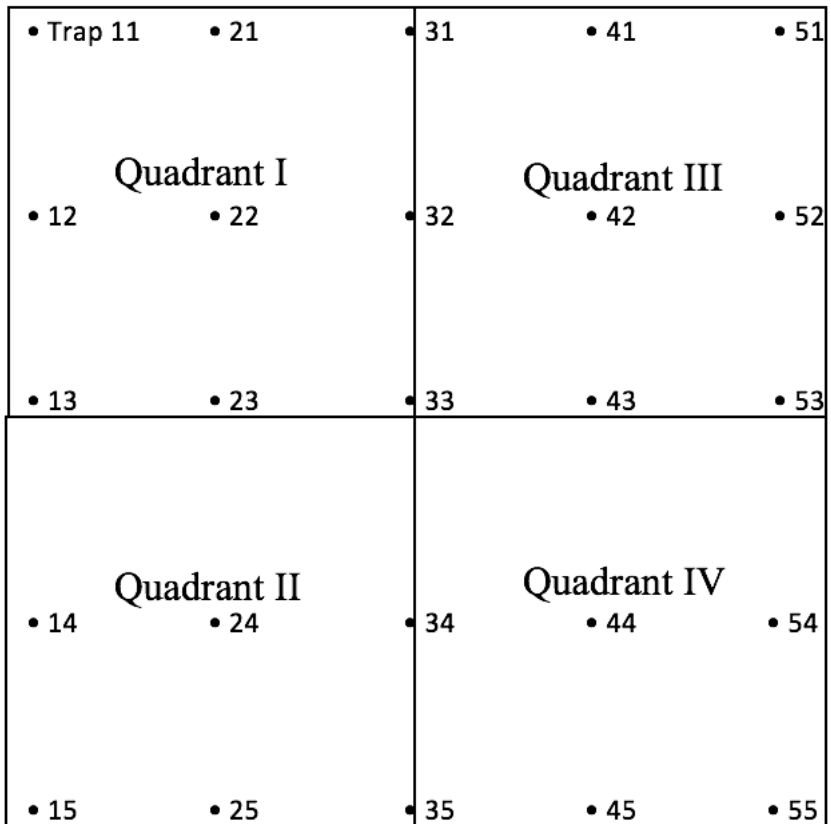
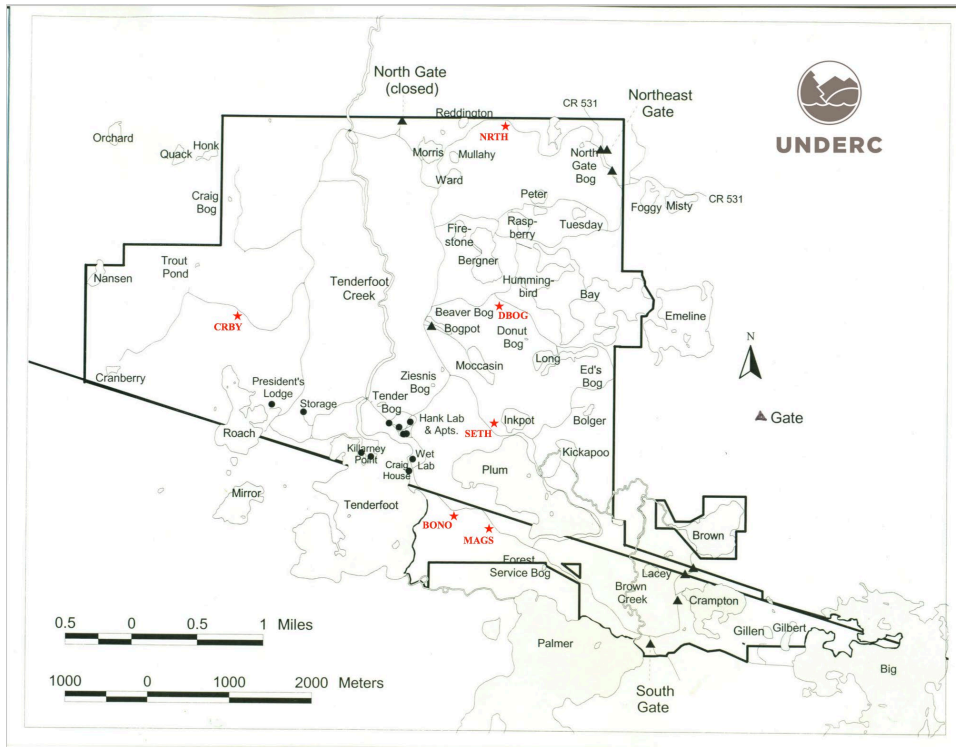
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FIGURE LEGENDS

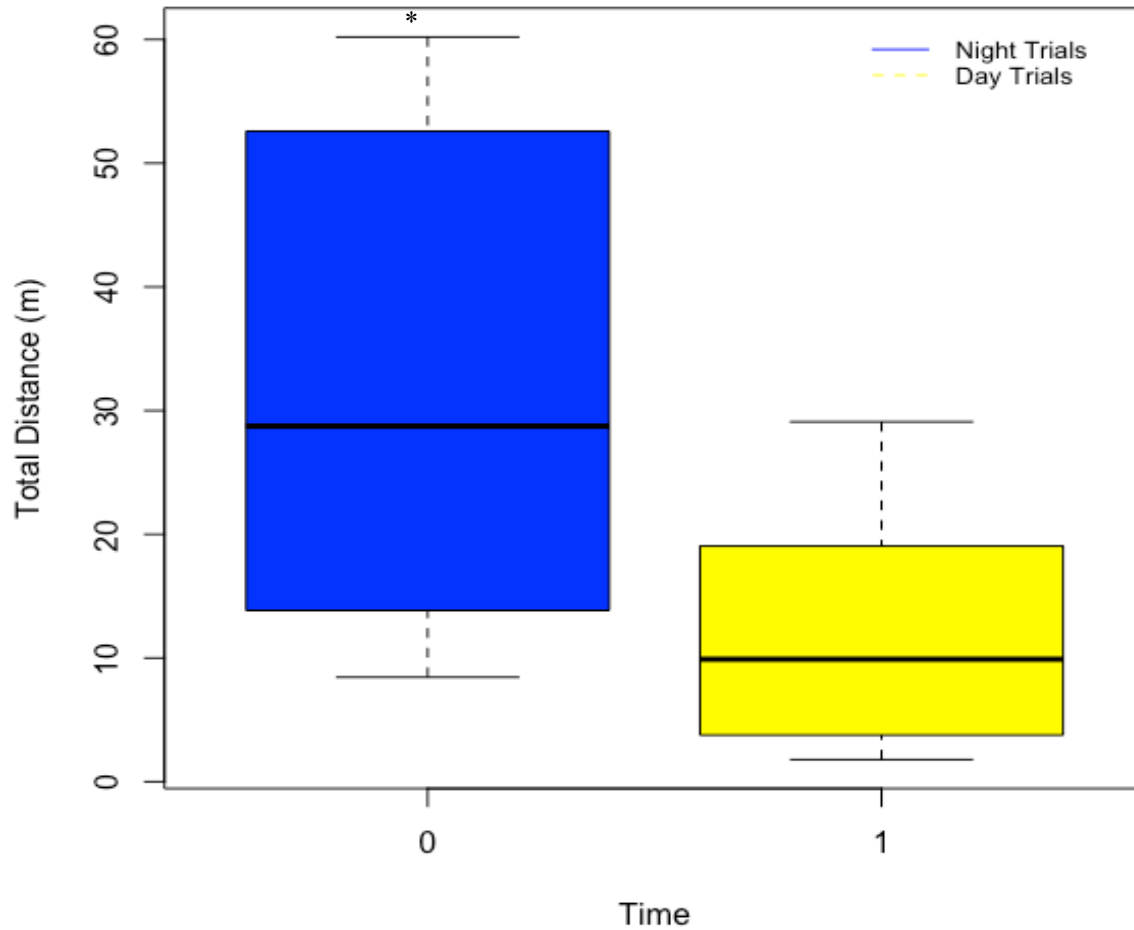
Figure 1. The trapping grid and release locations of the Peromyscus maniculatus used in this study. All contained a mixed hardwood forest with little to no understory and shrub layer.

Figure 2. A “full” trapping grid of 5 x 5 traps with 15 m spacing split into 4 quadrants (I-IV), marked with traps to further display the idea of the trapping grid. Half trapping grids would contain only quadrants I and II or I and III. The center points for each quadrant are as follows: I – 22, II – 24, III – 42, IV – 44. The center points were used as common release points for the Peromyscus maniculatus tracked in this study.

Figure 3. The total distance traveled of Peromyscus maniculatus in night and day trial settings. The night trial ($32.375 \text{ m} \pm 7.450$) deer mice traveled significantly farther than the day trial mice ($12.040 \text{ m} \pm 3.576$; $\chi^2 = 4.4118$, $df = 1$, $P = 0.036$), likely due to behavioral differences in avoiding visual detection by predators.



TOTAL DISTANCE TRAVELED



APPENDIX

Trial	Mouse Tag	Species	Time	Date	Sex	Total Distance (m)	Total Deviations	Proportion on LL	Proportion on CWD	Home	Grid	Wet	Proportion exposed
1	8223	Pman	1	7/12/19	1	13.4	28	0.30	0.70	1	BONO	0	0.01
2	7505	Pman	1	7/12/19	0	3.085	13	0.76	0.24	1	BONO	1	1.00
3	8107	Pman	0	7/12/19	1	14.776	26	0.62	0.38	1	BONO	1	0.99
4	8225	Pman	0	7/12/19	1	12.912	29	1.00	0.00	1	BONO	1	0.53
5	8227	Pman	0	7/13/19	0	8.445	12	0.92	0.08	1	BONO	0	0.66
7	8255	Pman	1	7/15/19	0	24.072	22	0.61	0.39	1	DBOG	0	0.60
8	8253	Pman	0	7/15/19	1	60.202	50	0.86	0.14	0	DBOG	1	0.97

9	8257	Pman	1	7/16/19	1	4.451	10	0.89	0.11	1	NRTH	0	0.96
10	8135	Pman	0	7/16/19	1	18.058	16	0.91	0.09	1	DBOG	0	0.85
11	8269	Pman	1	7/17/19	0	1.788	7	0.84	0.16	0	SETH	0	1.00
12	8277	Pman	1	7/17/19	1	6.401	10	0.90	0.10	1	MAGS	0	0.88
13	8271	Pman	0	7/17/19	1	51.928	50	0.93	0.07	0	MAGS	0	0.94
14	8285	Pman	1	7/19/19	1	29.096	25	0.72	0.28	1	CRBY	1	0.79
15	8137	Pman	1	7/19/19	1	14.026	11	0.75	0.25	1	CRBY	1	0.71
16	8281	Pman	0	7/19/19	1	39.428	48	0.53	0.47	1	CRBY	1	0.55
17	8287	Pman	0	7/19/19	1	53.251	41	0.37	0.63	0	CRBY	1	0.50

Collection of all data; where Pman is *Peromyscus maniculatus*; in “time” 0 is night and 1 is day; in “sex” 0 is female and 1 is male; in “Proportion on LL” where LL is leaf litter; in “home” where 1 is the trial ended at a definite location and 0 there was no discernible end location; and in “wet” where 0 is dry and 1 is damp conditions.