

Using Noninvasive Track-Based Monitoring Techniques to Estimate Small Mammal Activity Rates

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Introduction

Live trapping is a traditional technique for studying small mammal population and community ecology (Loggins et al. 2010) and is an essential component of mark-recapture population sampling, the most common method for estimating species abundance in a specific area (Clark et al. 2007, Glennon et al. 2002). Live trapping allows researchers to monitor age,

sex, size, and body condition of targeted mammals, but trapping can be time consuming and cost-restrictive (Glennon et al. 2002). It also presents physical risks to both the trapper and the trapped mammals, including exposure to infectious diseases such as Hantavirus (Drennan et al. 2013), which is transmitted through feces, urine, and saliva. Moreover, the Institutional Animal Care and Use Committee permits required for live trapping restrict a researcher's flexibility in developing a mammal-sampling protocol (Sikes et al. 2011).

Given these concerns, we considered noninvasive methods of monitoring small mammals. The use of noninvasive mammal-monitoring tools, such as camera traps and track tubes, has become increasingly common in wildlife biology. Motion-triggered trail cameras capture images (Brown et al. 2009) that document species identity and aspects of behavior and sometimes allow recognition of individual animals (Foster et al 2011). Cost restrictions, however, may limit the use of trail-cams in large-scale projects (Foster et al. 2011).

In this study, we explored a noninvasive track-collecting technique to monitor small mammal activity. Track tubes record the presence of animals through the tracks they leave on media inside food-baited tubes (Glennon et al. 2002). Attracted by the scent of the bait, animals enter a tube, pass across an inkpad, and leave ink spots on a track-recording medium. Ideally, the spots form recognizable, species-specific paw prints. Investigators have recorded tracks on media such as sand, smoked kymograph paper, talc-coated plates, ink-coated tiles, carpenter's chalk, acetate sheets coated with a graphite-alcohol-oil mixture, and clear contact paper (Connors et al. 2005). Previous studies have also evaluated several types of bait, including oat grains (Connors et al. 2005), a mix of peanut butter and oatmeal (Drennan et al. 1998), and black sunflower seeds (Loggins et al. 2012).

As a basis for estimating species abundance or detecting population changes, track based monitoring is not as straightforward or statistically effective as live-trapping techniques (Drennan et al. 2013). It is difficult to quantify the number of individuals that have visited a track tube, and, due to imperfectly formed or overlapping paw prints, track tubes are less likely than live trapping to permit species identification. However, track tubes are relatively inexpensive (less than \$2 per tube) and lightweight (Glennon et al. 2002), and they reduce both the disturbance of targeted mammals and the likelihood of exposing researchers to animal borne disease (Clark et al. 2007).

2

Track tubes are used in small mammal studies to monitor home range distributions, to record occurrences, especially for low-density species, and to estimate a relative index of population size and change (Drennan et al 2013). The purpose of our study was to explore the effectiveness of two types of track tubes in measuring the activity of small mammals in two distinct habitats on the property of Keene State College: a riparian woodland and a transitional field dominated by forbs and small shrubs. Riparian woodlands often feature a high insect abundance, soft soils for burrowing, and moist microclimates, while their structural complexity are often associated with high biodiversity (Vermont Fish and Wildlife Department 2015). We

hypothesized that the structural complexity and vertical dimensions of the riparian woodlands would create more shelter, provide more ecological niches, and support greater mammal abundance than the transitional field, leading us to predict a greater track density in tubes left in the riparian woods than in the transitional field. Among the small mammals that might enter track-tubes in the riparian zone are Eastern gray squirrels (*Sciurus carolinensis*), red squirrels (*Sciurus vulgaris*), white-footed mice (*Peromyscus leucopus*), meadow voles (*Microtus pennsylvanicus*), and pine voles (*Microtus pinetorum*). In the transitional field, we expected find prints of meadow jumping mice (*Zapus hudsonius*), white-footed mice (*P. leucopus*), and possibly house mice (*Mus musculus*) (Godin 1977).

We used track tubes of two different sizes for this study. Small track tubes had cross sectional dimensions of 6.4-cm (w) x 6.4-cm (h), while large track tubes, measuring 12-cm (w) x 12.5-cm (h), were both more spacious and more open to the environment. We hypothesized that smaller mammals would favor tighter spaces for greater protection from predators, leading us to expect more activity in small track tubes than in large track tubes.

Study Area

The transitional field lies on sandy soil south of New Hampshire Highway 101 near Keene State College's athletic complex. The sampling area, approximately 40 meters wide and 285 meters long, is oriented along a north-south axis and is crossed by a paved entry road about 60 meters from its north end. The field contains various bunch grasses but is dominated by goldenrod (*Solidago* spp.), milkweed (*Asclepias* spp.), silky dogwood (*Cornus amomum*), and sumac (*Rhus* spp.), which by late September have reached a height of about 1.4 meters. The field is bordered by shrubs on the north end, by a frequently mowed grassy cross-country running path on the southern and western edges, and by a wooded wetland on its eastern side. Utility lines run over the center of the field from north to south supported by regularly spaced wooden poles.

Keene State College mows the field annually at the beginning of November. Mowing maintains the early successional vegetation preferred by some species of small mammals but may also discourage their use of this area (Slade et al. 2006). In 2014, mowing was delayed until November 25th to facilitate our study.

3

The riparian habitat is composed of mixed deciduous and white pine woodland bordering the western bank of the Ashuelot River, forming a complex, 3-dimensional band of forest. Foliage and understory densities are greater in the spring and summer and decline in the fall. Floodplains dominate this zone, but flooding did not interrupt our study. The Ashuelot River runs generally north to south through southwestern New Hampshire for approximately 64 miles before emptying into the Connecticut River (New Hampshire Department of Environmental Services 2014).

Ashuelot River ecosystems are subject to developmental and recreational pressures. The Ashuelot River watershed is included in the Silvio O. Conte National Fish and Wildlife Refuge Act passed in 1991 to conserve, protect, and enhance the diversity of species that exist within the entire Connecticut River watershed (New Hampshire Department of Environmental Services 2014). The Ashuelot River Corridor Management Plan (2006) states that management is essential to ensure protection of legitimate community interests along the river, and to protect its existing natural resources. Ashuelot river conservation priority issues include land conservation, protection of ground water and tributary streams, preservation of agricultural land, and prevention of wetland loss, soil erosion, and floodplain development (Ashuelot River Local Advisory Committee 2006). The plan emphasizes the conservation of plant and animal habitat and the maintenance of natural riparian buffers by protecting riverbank forests.

Methods

Small mammal population sampling commonly involves use of grid- or a line-transects (Glennon et al. 2002). Our study sites were too small for grid sampling. Therefore, we placed 10 sampling stations along a linear route in each habitat, separating consecutive stations by a 37.8 m gap roughly equal to the average distance between center points of home territories of the Eastern chipmunk (1,600 square meters), as determined by Michigan researchers (Mores et al. 1980). We initially placed stations in both the woodland and transitional field strictly according to this protocol. We tied flagging tape to vegetation 1.5 m to 2.5 m above the ground at each station and drove plastic tent stakes into the ground to identify the stations. We sampled both habitats concurrently from October 6 to November 13, 2014, before the onset of winter snow and before freezing of the soil.

Transitional Field Habitat Stations- We established a north-south line transect along the mid point of the field equidistant from a cross-country running path (western border) and woody margin (eastern border). This transect ran directly below a set of utility power lines. The first six stations were placed at 37.8-m intervals progressing north toward a paved entry road, with Station 1 located 18.2-m north of the southern edge of the field. Initially, we positioned stations 7-10 in the section of the field north of the entry road, with Station 7 located 18.2-m north of the paved road. We eliminated Stations 9 and 10, however, after another research team applied Roundup® herbicide around them; we replaced them by adding Stations 11 and 12 near

4

the wooded eastern edge of the field south of the entrance road. Station 11 was equidistant from stations 2 and 3, and Station 12 was equidistant to station 4 and station 5.

Riparian Habitat Stations- A preliminary survey of the woods along the west bank of the Ashuelot River led us to place ten sampling stations in a continuous band of forest whose width (from river on the east to grassy running path on the west) ranged from 3-m to 75-m. The stations were located near the steeply inclined, vegetation-free river bank, set back from the

bank by 1.5-m to 4.5-m. Station 1 was placed at the north end of the transect and Station 10 at its southern terminus, with all stations spaced at 38-m intervals. The river meanders, so the transect was not straight, but it lay entirely within the woods, not far from the river's edge.

Sampling Procedure

Track tubes were constructed from 30.5-cm-long sections of white vinyl rain gutter, assembled as described by Glennon et al. (2002). Large track tubes, formed by apposing two open-faced, U-shaped gutters to form an enclosed tube, were 12.5-cm high; small track tubes, each formed from a single square-shaped rain gutter, were 6.4-cm high. At the mid-point of each tube, we fixed a short section of bisected circular 1" PVC piping to serve as a bait tray, which we filled with approximately 1.5 g of peanut butter. At each end of the tube we fixed a felt pad dampened with ink (a 3:1 mixture of light Fisher mineral oil and carbon black). We taped a sheet of transparent contact paper between the central bait tray and each inkpad; the dimensions of these sheets were 12.5-cm (l) x 6.9-cm (w) for large track tubes and 12.5-cm (l) x 6.4-cm (w) for small track tubes. Thus, each tube contained one bait tray, two ink pads, and two sheets of contact paper. A detailed description of track tube construction and cleaning will be provided upon request.

We sampled each station for three consecutive days (one cycle), to allow animals to habituate to the track tubes. Tubes were placed at three to four stations, separated by two unsampled stations. This separation was intended to reduce the likelihood that a single animal would visit multiple track tubes during a sampling cycle (and thus lead to overestimates of mammal activity). Typically, cycle 1 included Stations 1, 4, 7, and 10; cycle 2 included Stations 2, 5, and 8; cycle 3 included Stations 3, 6, and 9. The first days of the three cycles were staggered by one day. We checked track tubes every 24 hours: If ink spots were seen on the contact paper, we replaced that track tube with a fresh one of the same size ("large" or "small") for the duration of the 3-day cycle. In the absence of ink spots, a single track tube occupied a station for three days. If new ink spots appeared each day at a station, three different tubes were used during that cycle. After completion of cycle 3, we returned to cycle 1. In each cycle, we deployed both types of track tubes in a pattern that assured equal usage of large and small track tubes at each station and in each habitat. For example, in the first round of cycle 2, we used two large track tubes and one small track tube. For the second round of cycle 2, we used two small track tubes, and one large track tube.

5

The contact sheets were so heavily trafficked and prints overlapped to such a degree that few individual paw prints could be recognized. Therefore, to estimate small mammal activity we quantified the number of ink spots per track tube. Theoretically, each ink spot corresponded to an individual toe or pad impression. We laid a transparency with an 80-cell grid over each contact sheet and counted the number of ink spots in ten randomly selected cells, using the random number generator on Excel 2013 to generate a unique set of ten target

cells for each contact sheet. We counted all ink spots of all sizes, whether light or dark, including large smudges and small distinct spots over or under smudges. We did not count peanut butter stains or leaf debris.

For each type of track tube, we defined the small mammal activity rate at a sampling station in terms of the relationship between number of ink spots and total exposure of track tubes to potential mammalian visitors. Specifically, for each station and type of track tube we divided the sum of spots counted on all track tubes (two contact sheets per track tube) by the sum of “track-tube days.” One “track-tube day” equaled the exposure of one track tube for 24 hours. Total exposure of our track tubes (the sum of all days of exposure of all track tubes at all stations) was 127 track-tube days, a numerical measure of the total opportunity for track collection.

We used the statistical application JMP for data plotting and analysis of variance.

Results

Fifty-one track tubes were exposed in the riparian habitat for 63 track-tube days, while only twenty-five track tubes were needed to cover 64 track-tube days in the transitional field habitat, owing to less frequent tube replacement. Despite equal exposure, fewer track tubes placed in the field collected ink spots than track tubes in the riparian woodland. Over the sampling period, the fraction of track tubes that collected ink spots in the transitional field was only 0.12 (three track tubes: one large and two small), while every track tube placed in the riparian woodland yielded ink spots. Thus, simple observation revealed a large difference in apparent small mammal activity between the riparian woodland and the transitional field.

The high frequency of track-tube days without ink spot in the transitional field strongly skewed the data to the right, precluding a standard analysis of variance. Initial graphical analysis suggested no interaction between habitat type and tube size but to formally assess the influences of track-tube and habitat differences, we conducted a non-parametric one-way Kruskal-Wallis analysis of variance for our sample data. The results of this analysis are illustrated in Figure 1.

Regardless of tube size, many more ink spots were collected in the riparian woods than in the field. Large track tubes deployed in the riparian woodland yielded a mean rate of activity of 447.5 sample spots/track-tube day, while large track tubes deployed in the transitional field recorded a mean rate of activity of 10.2 sample spots/track-tube day (Figure 1). Ink spot data from large track tubes revealed significant variance in activity rate between habitat types (df =

1; $P = 0.0011$). Data from small track tubes in the riparian woodland showed a mean small mammal activity rate of 370.3 spots/track-tube day, while the rate for transitional field was around 8.2 spots/track-tube day (Figure 1). This variation in small track tube activity rate between habitat types was also strongly significant (df = 1; $P = 0.0006$). On the other hand, there was no significant variance in activity rates between the two tube sizes in either habitat

(riparian woodland: $P = 0.6338$; transitional field $P = 0.9165$).

Finally, we evaluated the spatial distribution of ink spots on contact sheets. Using the 80-cell transparency grid, we determined for each contact sheet that bore ink spots the fraction of the 80 cells that contained ink spots. With regard to those contact sheets on which animals left ink spots, the mean fraction of grid cells bearing ink was greater for the riparian woodland than for the transitional field, regardless of track-tube size. For large track tubes in the riparian woodland the mean fraction of cells that bore ink was 0.75, *versus* 0.57 for large track tubes from the transitional field. Similarly, the fraction of the contact sheet grid that bore ink in small track tubes was 0.76 for the riparian woods but only 0.66 in the transitional field. Figure 2 illustrates the wider distribution of ink spots on contact sheets exposed in the woods compared

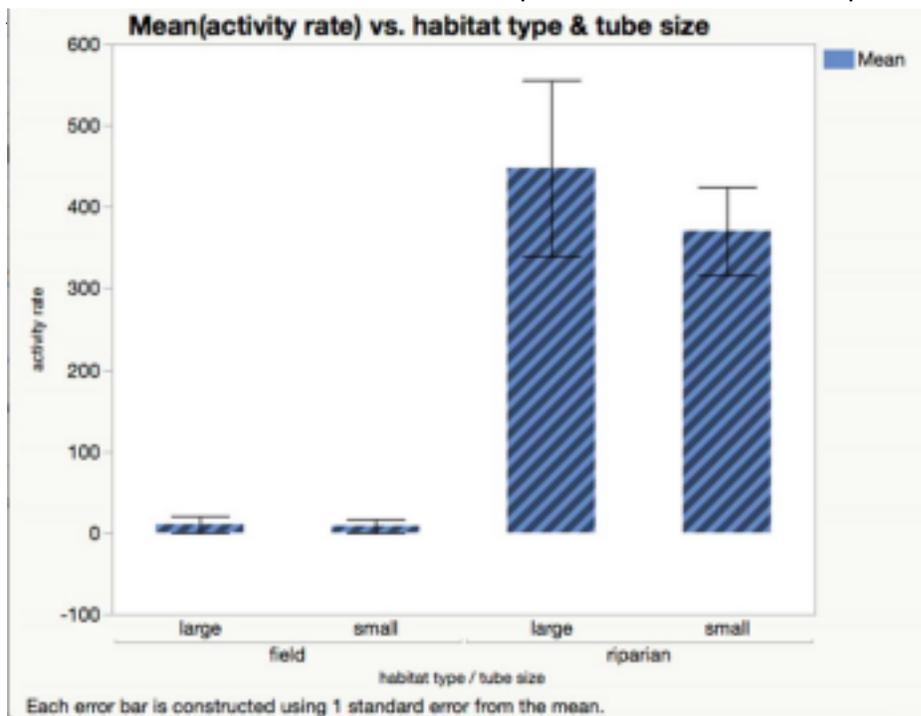


Figure 1- Effect of habitat type on variance in activity rate between large and small track tube sizes. Effect of tube size on variance in activity rate between the riparian woodland and transitional field habitats. Error bars represent the standard error of the mean.

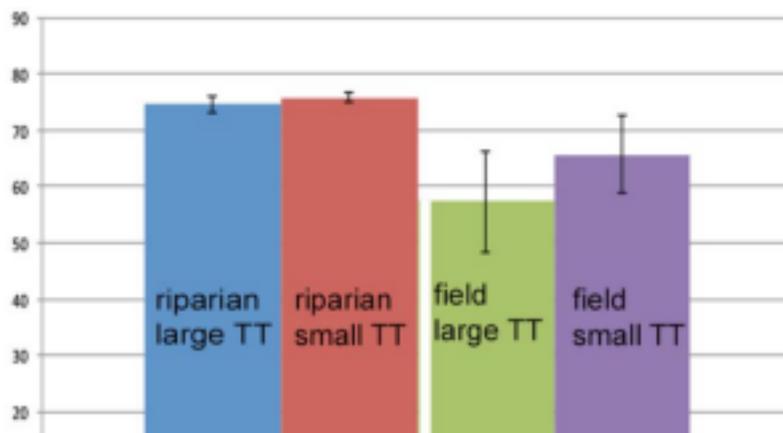


Figure 2- Portrays the

mean fraction of contact sheet surface that bore ink spots in both habitat types for both tube sizes. Error bars represent the standard error of the mean.

Discussion

Our track tubes efficiently recorded ink spots from the paws of visiting mammals, but imperfect and overlapping prints made analysis of the ink spots difficult. We were not able to identify species or distinguish tracks from ink smudges. Moreover, we cannot specify the number of individuals that visited each tube, but our noninvasive track-based technique revealed a striking difference in the apparent rates of small mammal activity in the two habitats.

We speculate that the high rate of small mammal activity in the riparian woodland resulted from a greater abundance of mammals residing there than in the transitional field. In New England, many small mammal species feed primarily on the seeds of trees and shrubs, an activity that enhances seed dispersal and sustains a mutualistic relationship. Since meso predators and apex predators, such as the red fox (*Vulpes vulpes*) and the red-tailed hawk (*Buteo jamaicensis*), depend on small mammals as primary prey, robust populations of small mammals generate a bottom-up ecological pressure that fosters the persistence of riparian woodland communities. Our data is consistent with the notion that the woodlands surrounding the Ashuelot River support greater mammalian abundance than the adjacent transitional field.

What about mammalian species diversity? We speculate that the primary source of the ink spots in our track tubes was the white-footed mouse (*P. leucopus*). This species prefers woodland habitat with white pines, hemlocks, and oaks (Godin 1977), all of which are present along the Ashuelot River. White-footed mice typically nest in trees flanking the edge of such woods (Godin 1977). Some of the ink spots may also have been left by meadow voles (*M. pennsylvanicus*). Meadow voles are extremely abundant in New England and prefer to inhabit areas near streams or lakes (Godin 1977). We visually identified both species during our field operations. White-footed mice leave prints about 1.0-cm wide x 0.8-cm long (front) and 1.0-cm

8
wide x 1.0-cm long (rear) (Halfpenny 2001). Meadow voles usually leave more elongated prints about 1.0-cm wide x 1.0-cm long (front) and 1.0-cm wide x 1.3-cm long (rear) (Halfpenny 2001). Both print dimensions are compatible with the distribution of ink spots on our contact sheets,

but the extreme density of spots on each marked contact sheet precludes definitive species identification.

Several factors could have led to the lower apparent rate of small mammal activity in the transitional field. The annual fall mowing may deter small mammals from staying there. The structural simplicity of the habitat and lack of large trees probably rendered it less habitable for cavity-dwelling rodents. Interestingly, the field contains many slugs. We did not determine the species of slug found in our track tubes, but we recovered approximately three slugs per track tube at each 24-hour checkpoint. We confirmed that the slugs consumed the bait while inside of the tube. Although some rodents eat slugs, some small mammals may avoid entering tubes or eating bait covered with their slime.

At only two of ten stations in the transitional field did track tubes collect ink spots. Station 4 was directly adjacent to a pair of tall wooden utility poles. Mice often take refuge near the bases of trees (Godin 1977); perhaps the base of a utility pole may also provide shelter, which would explain activity at this particular station. Station 11 was located near the wooded eastern margin of the field. It is possible that a small mammal in this woody tract caught the scent of the track tube bait and traveled into the field to feed.

Mammal activity rates did not vary significantly between track tubes of different sizes. As our data do not suggest that small track tubes were more attractive than large track tubes to small mammals, there is no need to hypothesize that they provide greater security to visiting animals.

Conclusions

Our track tubes recorded the activity of small mammals in two habitat types at Keene State College. There was a much higher rate of activity in the Ashuelot River woodlands than in a nearby transitional field, suggesting that these woodlands sustain a larger population of small mammals than the transitional field. Large and small track tubes yielded similar results, reinforcing the impression of important habitat differences. In separate trials on other parts of the campus, we observed that larger animals, such as woodchucks (*Marmota monax*), could not reach the bait in the small track tubes. Therefore, larger track tubes might be more suitable than small track tubes for studies that target a wider range of animal species.

For future studies, we suggest a few refinements of methodology. First, a longer sampling season or larger study area would yield more data points and increase the statistical power of our data analysis. Second, our study was initially exploratory, but any follow-up study should adopt a sampling schedule and baiting technique that might generate normally

distributed data, in order to permit a two-way ANOVA and corresponding F-tests. We conjecture that an overuse of peanut butter led to multiple visits to track tubes and the

saturation of contact sheets with ink spots, rendering the paw prints indecipherable. In most cases, the peanut butter bait was completely removed by animals that entered the track tubes. Perhaps if we use less bait, visiting mammals would spend less time feeding and walking inside the track tubes. Shorter visiting periods should lead to fewer ink spots and more distinct and identifiable tracks. We would like to learn which species visited the track tubes, so placing trail cameras near some of the them might allow definitive species identification and a greater understanding of the biodiversity of habitats such as the Ashuelot riparian woodlands.

Acknowledgements

Niko Brown, an undergraduate student at Keene State College, assisted in both fieldwork and data analysis. Professor Bergman conceived of the project and provided supervision and material support. I thank Dr. Cynthia Hays, Assistant Professor of Biology at Keene State College, for providing invaluable statistical advice.

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