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THE USE OF FLUORESCENT POWDERED PIGMENTS AS A TRACKING TECHNIQUE FOR SNAKES

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***Abstract.*—We describe a new technique for tracking snakes that is an alternative to radio-transmitters and thread trails. We coated the bodies of three species of garter snakes (*Thamnophis*) in fluorescent powder, then followed and marked the trails with a UV light at night. The use of a UV light allowed us to see very detailed paths left by the snakes. This technique was effective for snakes > 10 g and we tracked some individuals > 200 m. Because a portion of the paths ended in burrows, this technique may prove useful for locating hibernacula sites. Fluorescent powder tracking is a useful tool in determining where and how snakes move in a localized habitat.**

Key Words.—fluorescent powder; radio-telemetry; snake; technique; *Thamnophis*; thread trailing; tracking

INTRODUCTION

Fluorescent powder tracking provides an exact record of movement (Stark and Fox 2000) and is therefore useful in discovering how an animal moves through its environment. Using fluorescent powder is also non-invasive, inexpensive, and can be performed successfully with little training. Application of fluorescent powder on body surfaces is an underused method for obtaining data on the movement patterns of reptiles. The technique was originally developed for tracking small mammals (Leman and Freeman 1985) and has been used to obtain data on population sizes (Hubbs et al. 2000) and habitat use (Corbalan and Debandi 2009). Powder tracking has been used for tracking anurans (Graeter and Rothermel 2007), urodeles (Roe and Grayson 2008), turtles (Blankenship et al. 1990) and lizards (Dodd 1992; Stark and Fox 2000), but there are no reports of this technique being used for snakes.

Radio transmitters and bobbin thread trailing have been used previously to track snakes (Tozetti and Martins 2007; Dorcas and Wilson 2009). However, both of these methods have shortcomings. In contrast to a dusting of fluorescent powder, carrying the mass and bulk of a radio transmitter or spool pack poses an energetic challenge, particularly for small snakes. Using radio transmitters typically involves invasive surgery and requires training. Also, the use of general anesthetic poses a risk of death to the animal if not handled properly (Holtzman et al. 2002). The benefit of transmitters is the possibility for long term tracking. As for thread tracking, animals that enter burrows or move through dense vegetation run the risk of getting the spool pack caught, thus risking injury and alteration of natural movement patterns (Lemckert and Brassil 2000). Fluorescent powder tracking provides similar information as thread trails, but with less risk of significantly altering snakes' behavior. In this study, we examine whether fluorescent powder can be used to track three *Thamnophis* snake species in Alberta, Canada.

MATERIALS AND METHODS

We caught and tracked 19 garter snakes, representing three species (10 *Thamnophis sirtalis*, five *T. radix*, one *T. elegans,* and three individuals that were most likely *T. sirtalis,* but identification records were lost) in ravine habitat along Whitemud Creek, a tributary of the North Saskatchewan River within the city limits of Edmonton, Alberta, Canada. We captured these snakes during visual surveys in daylight hours between 0900 and 1500 (6 May – 4 June 2009). All capture sites were located within 20 m of a water source (stream or wetland), but surrounding vegetation was variable. In forested areas, Trembling Aspen (*Populus tremuloides*) and White Spruce (*Picea glauca)* dominated. Other non-forested areas were covered mainly by grass and forbs and/or supported shrubs mixed with herbaceous plant species. Many of the snakes we tracked either moved along the top a bluff exposed soil caused by erosion was another common habitat feature. We captured all snakes within 30 m of two known hibernacula sites. Following hand-capture, we held snakes in 5 or 10 L containers. Prior to powder application, we recorded snout-vent length (SVL) and weight, and we marked the animals with a cautery iron following procedures described in Winne et al. (2006) for individual identification. We held snakes from 20 min to five days prior to tracking. Most individuals were held one hour or less.

We powdered the snakes by placing their bodies in a plastic bag containing about 2.5 cm of powder, and

FIGURE 1. Common Garter Snake (*Thamnophis sirtalis)* immediately following application of orange fluorescent powder. (Photographed by Murdoch Taylor)

gently tipping the bag in various directions to coat all sides of the snake (Fig. 1). Powdering was carried out away from the capture/release area. During powdering, the snake's head was kept out of the bag to ensure that no powder got into the eyes or mouth. The powder we used (approx. $10 US/lb, Radiant Color Series T1, DayGlo Color Corporation, Cleveland, Ohio) was either orange or green. Graeter and Rothermel (2007) suggested that green was among the best performing color (the other being chartreuse) followed by orange and pink. Our selection of color was based upon proximity to other snakes released the same day and to previous tracks that persisted in the environment. Thus we avoided confusion of individual paths, as suggested by Stark and Fox (2000). Once the powder was applied, we carried snakes to the site of release, still within the bag, and then removed and placed them on the ground. We released all snakes within 5 m of their original capture sites when the temperature was > 15° C; time of release varied from late morning to early afternoon.

Our documentation of powder trails was typically done approx. 36 h following release starting around 2300. This allowed time for snakes to move and sufficiently low light levels for proper sighting of tracks. We used a fluorescent industrial lamp (containing two 90 cm black light bulbs) that was connected to a portable, gas powered generator with a 100 m extension cord. The point where we released the snakes was marked with a wire flag, along with all locations that indicated a directional change > 10°, so as to create a detailed record of movement. We followed trails until powder could no longer be detected because it had been depleted, the snake had entered water, or it had gone underground. The following day, we took our measurements using the flagged trails. We recorded the angle of path change from flag to flag with a compass and the distance between flags with a tape measure (referred to as "actual distance;" AD). We also recorded the straight line distance (SL) between the start point (site of release) and the end point (where powder was last visually detected). Data were then compiled in an ArcGIS program (Environmental Systems Research Institute, Redlands, California, USA) to create a detailed reconstruction of the paths.

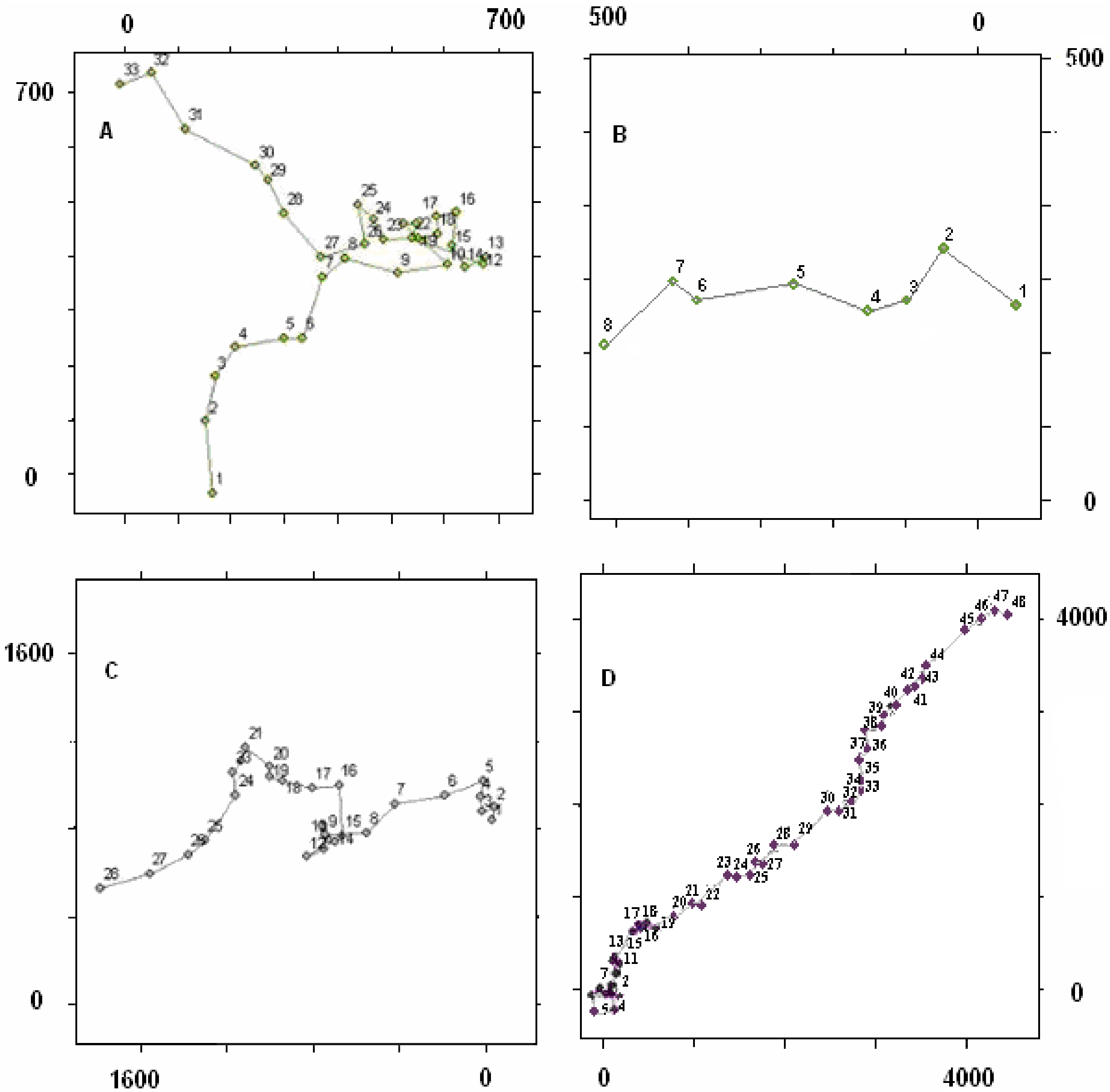
To determine whether snake body size was correlated with track length, we used Spearman's correlation on snake mass and the AD of the powder trail. We also used Spearman's correlation to determine if mass and SVL were correlated. To determine if the beginning of the track was different from the remainder, we compared the ratio of AD:SL for the first six turns (> 10° change in direction of path) to the AD:SL ratio for the second set of six turns. This ratio reflects the amount of linear progress made by the snake; the higher the ratio, the more convoluted the path. We chose a series of six turns as the unit of comparison because preliminary analysis of the difference in AD:SL ratios indicated that shorter series of turns had less distinguishing patterns (less difference in AD:SL ratios between the first set of turns and the second) than the comparisons of six turns. We calculated this ratio for each set of turns (first six turns and second six turns) for each snake (excluding the seven shortest tracks; AD < 30.2 m, SL < 23.6 m) and carried out a two-sample t-test that assumes unequal variances for the data. We natural-log transformed the data to achieve normality prior to analysis.

We used a one-way ANOVA on track length data to determine if paths remained fairly straight after the initial release (i.e., whether or not comparisons of AD:SL ratios for sets of six turns differed significantly along the rest of the track). To obtain values for this analysis, we broke up an individual snake's track into a series of six turns, then (starting at the second set of six turns) the AD:SL ratio was determined for each set of six. We repeated this process for each track, then the sets of turns were grouped across all snakes' tracks. Therefore, the second set of six turn's AD:SL ratio was grouped for all snakes, the third set of six turn's AD:SL ratio was grouped for all snakes, and so on for a total of four groups of sets (sets two through five). We then used a one-way ANOVA to compare these five groups with values consisting of individual snake's AD:SL ratio for a particular set of turns. As this analysis required long tracks, we only used the four longest snake tracks. For all tests, we defined α as 0.05.

RESULTS

Trails created by snakes started as distinct lines of powder, then degraded into patches of powder on vegetation, and eventually to small flecks. The tracks we obtained were highly variable (Fig. 2) in actual and straight line distance over all snakes tracked (3.08–203.4 m AD, 1.28–120.4 m SL; Fig. 3). Some tracks were shortened prematurely by snakes entering water (n = 4) or mammal burrows (n = 2), or shedding their skin (n = 2). We do not know whether application of powder could have actually triggered shedding.

We tracked snakes over a size range of SVL (17.3–70.1 cm) and mass (3.1–171 g; Fig. 2). The SVL and mass of snakes were highly correlated (r = 0.976, df = 18, P < 0.001). We found a significant correlation between the snake's mass (g) and the length of powder trail (AD) it produced (r = 0.846, df = 10, P < 0.001). Additionally, a clear difference existed between seven small snakes believed to be young-of-the-year snakes (< 10 g, King et al. 1999; mean AD: 11.94 ± 3.68 m SE; mean SL: 9.19 ± 3.07 m SE) and 13 older, larger snakes (> 10 g; mean AD:



cks. The numbers along these trails indicate the position of turns that were greater than 10°. These points were flagged and measurements taken between flags. Examples: (A) detailed record, illustrating variable behavior that can be recorded using fluorescent powder tracking, (B) a short trail left by a small, 3.6 g snake, (C) straightening of a trail after six turns > 10°, and (D) a long trail left by a large, 126.8 g snake.

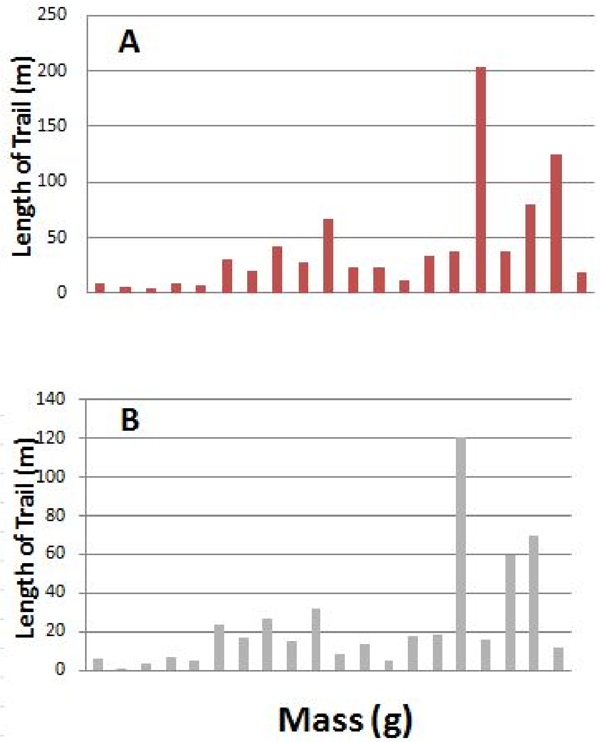


FIGURE 3. Length of fluorescent powder trails left by three species of garter snakes (10 *Thamnophis sirtalis*, five *T. radix*, one *T. elegans*, and three snakes lacking species records [likely *T. sirtalis*]). Individuals' performances are organized along the X axis left to right based on increasing body size, smallest mass (3.1 g) to largest mass (171.6 g) of snake. Actual distance (A) is the sum of distances measured between turns of 10 or more degrees. Straight line distance (B) is the linear distance from the point where the snake was released to the point where powder was last detected.

56.07 ± 14.96 m SE; mean SL: 32.00 ± 9.09 m SE). Trails of the assumed juvenile snakes never exceeded 30.2 m (AD) and 23.7 m (SL), whereas larger snakes often created longer trails (AD range: 11.5–203.4 m; SL range: 5.62–120.4 m).

The behavior of snakes immediately following release appeared atypical compared to the rest of the track, both visually and based on detailed measurements. This was documented when we compared the ratio of AD:SL between the initial six turns after release and the next six turns of the trail for 13 snakes. There was a significant difference in the AD:SL ratio between the first six turns (mean = 2.11 ± 0.36 m SE) and the next six turns (mean = 0.97 ± 0.11 m SE; t = 3.66, df = 25, P < 0.001), which suggests that snake movements became more linear. To determine if this increase in linear progress was characteristic only of the first set of six turns compared to the second set of six turns, we did similar comparisons for the entire length of the longest paths (n = 4). The AD:SL ratios did not differ significantly among groups of six-turn sets further along the trials (F = 2.84, df = 3, 12, P = 0.082) indicating that as animals acclimatized following release; their paths became and remained fairly straight (Table 1).

TABLE 1. Ratio of average actual distance (AD) to straight line distance (SL) for the four garter snakes with the longest trails created by fluorescent powder tracking. Set comparison refers to ratios for one set of six turns compared to the next set of six turns.

Set comparison	Initial set of six points AD:SL ratio	Following set of six points AD:SL ratio	Difference between ratios
1–2	3.15	1.24	1.92
2–3	1.24	1.01	0.22
3–4	1.01	0.68	0.33
4–5	0.68	0.40	0.28

DISCUSSION

We found that using fluorescent powder to track garter snakes offers a useful technique for studying movement patterns and orientation in relation to habitat features. The change in behavior, indicated by our comparison of the AD:SL ratios between sets of turns, suggests that handling or acclimatization to the environment affected snake movement patterns, but only temporarily. Dodd (1992) reported that 19 of 40 tracks created with fluorescent powder by Six-Lined Racerunners (*Aspidoscelis sexlineata*) were short (< 10 m) and led directly to some form of shelter, presumably reflecting adverse responses to handling. Future applications of this technique on snakes should take into account the distinctiveness of initial movements (approx. first six turns), as they may be affected by handling or disorientation. Presumably after about 7 m of travel, garter snakes began to exhibit more typical behavior and move more in a single direction (Larsen 1987). This change in movement characteristics may have been due to snakes either recovering from stress associated with handling or first recognizing, then reorienting within, a familiar environment (e.g., Fig. 2D). Initial, convoluted movements could also represent attempts by snakes to remove powder from their skin. Movement in a consistent direction has been reported in studies using various methods, such as thread-tracking of Eastern Painted Turtles (*Chrysemys picta picta*), which showed that even in unfamiliar territory, movement was highly directional and remained consistent over the course of a track (Caldwell and Nams 2006).

Our results indicate that tracking with fluorescent powder is most effective in revealing short term, small scale habitat use by garter snakes. The lengths of tracks we documented (3–203 m AD) are comparable to other studies of amphibians and reptiles that used fluorescent powder as a means of tracking. For example, Graeter and Rothermel (2007) reported tracking multiple species of amphibian (*Ambystoma opacum, Anaxyrus terrestris, Lithobates sphenocephala*) for distances of 2 to 350 m. Dodd (1992) recorded track lengths between 1 m and 71 m for *Aspidoscelis sexlineata*. Stark and Fox (2000) used this technique on another squamate species, Texas Horned Lizards (*Phrynosoma cornutum*), and obtained tracks varying in length from 5 m to 225 m. Our track distances are within ranges of distances reported for garter snakes based on other tracking methods. Larsen (1987) reported that radio-tracked *T. sirtalis* in northern Alberta displayed daily movements of 50 m to > 1 km.

One advantage of powder tracking over radio telemetry is that the precise path of the animal is recorded, as well as the habitat features that it encounters and actually uses. This type of information would require constant visual contact with a snake being followed via telemetry, which could influence the snake's behavior. In contrast, powder tracking trails are similar to those left by thread trailing. However, as noted, a disadvantage of thread tracking is that the attached device can easily get entangled in surrounding vegetation and that the weight of the thread spool could be a constraint for tracking small animals. In addition, thread can easily be displaced prior to recording, whereas powder has a high resistance to disturbance, even remaining visible for two years after initially being deposited (Halfpenny 1992). The weight of fluorescent powder is negligible (< 0.5 g), less or equivalent to the smallest practical bobbin (approx. 1.7 g) or radio transmitter for implantation (approx. 0.65 g), and unlikely to affect distance or speed of movement. Although there are likely few adverse effects caused by powder application (Rittenhouse et al. 2006), researchers adopting this technique should be aware that application of fluorescent powder could make snakes more conspicuous to predators (Dodd 1992).

The effectiveness of fluorescent powder tracking may vary among and within groups of reptiles based on morphological characteristics. For example, we found that smaller garter snakes left shorter tracks than larger garter snakes, presumably because their smaller body surfaces collected less powder that dissipated over shorter distances. Stark and Fox (2000) similarly reported that juvenile *Phrynosoma cornutum* produced shorter tracks than adults. Garter snakes have fairly rough scales compared to non-keeled snakes, a feature that may increase the amount of powder that adheres to their bodies and its retention, thus improving the quality and length of track. Stark and Fox (2000) suggested that keeled scales of lizards (such as *Aspidoscelis sexlineata*) enhance powder tracks. Thus, this technique may not work well for smooth-scaled snakes (e.g., Pythonidae). Habitat can also affect tracking success. Movement through grassy and herbaceous vegetation, such as used by garter snakes, tends to leave the best powder trails (Stark and Fox 2000). However, all three *Thamnophis* species regularly traveled through shallow water, and this usually removed all powder and ended the record; 20% of our trails were affected by this behavioral pattern. Daily weather should also be taken into account as rain can reduce the visibility of the trails greatly (Dodd 1992). Of our tracked snakes, 10% ended up in tunnels or burrows, thus the use of fluorescent powder tracking may prove useful in finding hibernacula sites (Eggert 2002). Fluorescent powder tracking is proving to be an effective technique for tracking amphibians and reptiles, and we recommend its use for tracking snakes as part of ecological studies.

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