



Evaluation of Methods for Detecting Nonfluorescent Colored Flakes and Flake Persistence in Coyote Scats

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ABSTRACT

Effective markers are needed to investigate coyote behavior, especially in areas of feeding and bait acceptance. We evaluated three methods for detecting nonfluorescent colored flakes in coyote scats and the persistence of flakes in stored scats. Captive coyotes were offered tallow baits with 100 mg each of red, green, blue and a mixture of red and green flakes. Scats deposited daily for 5 days after bait consumption were examined for flakes on the surface, after crushing and after washing in nylon bags. Post-baiting, correct-colored flakes were detected for an average of about 2½ days in both surface and crushed-scat examinations and for nearly 4 days in washed scats, which was significantly longer. Preparing bags and washing must be done carefully. The flakes were persistent in scats — no color fading or color separation from the silver-colored bases was observed during 8 months of weathering and storage. The flakes made durable coyote-scat markers that were reliably detected with simple, inexpensive materials. The scat-washing technique described would probably be useful in detecting most inert particles delivered in baits.

INTRODUCTION

Animal marking techniques of various kinds are commonly employed for a variety of wildlife identification and management purposes (Stonehouse, 1978; Savarie *et al.*, 1992; Nietfeld *et al.*, 1994). Fall and Johns (1987) used

small colored flakes to mark rats at feeding points, and indicated that this marker would be well suited for mammals where scat examination was a routine study method.

Effective markers that are easily applied and economically detected are needed to investigate coyote (*Canis latrans*) behaviors, especially those related to feeding and bait acceptance. Baiting appears important in managing these predators and the diseases they carry, and will probably be used in delivering such things as oral vaccines and reproductive inhibitors. Inert particles would make good markers for coyotes or other animals with keen senses of taste and smell because they impart little or no flavor or odor that could influence bait acceptance. In addition to being inert, colored flakes offer other advantages; they are easy to detect with low-cost, readily available instruments, require no chemical treatment or analyses for detection and come in several colors.

A limitation of inert particle markers used in baits is their short persistence; they remain in the digestive tract for only a few days (Savarie *et al.*, 1992). Evans *et al.* (1971) found that inert aluminum paint pigment was detectable with microscopic examination for 4–6 days in nutria (*Myocastor coypus*) feces. How long colored flakes can be detected after ingestion by coyotes and persistence of flakes in stored coyote scats is not known.

We evaluated three methods of examining coyote scats for inert colored flakes to determine the most effective detection method and examined scats stored up to 8 months, to determine the persistence of flake color and integrity. Different colors of flakes were offered to coyotes in tallow baits, and scats deposited daily by each coyote were examined on the surface of whole scats, after the surface-examined scats were crushed, and after the crushed scats were washed in nylon bags. Washing scats in nylon bags was previously used to estimate coyote food intake (Johnson & Hansen, 1979).

METHODS

Nonfluorescent colored flakes (Glowable^R, originally developed for automotive paints) for the study were purchased from Metalflake, Inc., P.O. Box 950, Haverhill, Mass. 01830. Since the study ended, Metalflake, Inc. has gone out of business, however, similar kinds of particles that could serve as markers are available from other companies, see the *Thomas Register of American Manufacturers* (1995, 14945) under Glitter.

The Glowable^R flakes consisted of a colored coating over a silver-colored polyester film base. Flakes came in 10 brilliant colors, were flat,

rectangular and varied somewhat in size. A typical particle measured 442 by 374 by 34 μm ; there were about 131,000 particles per gram (Fall & Johns, 1987).

Test baits contained either a single color; bright red, Nile green, or royal blue, or two colors mixed together, bright red and Nile green. Each bait contained 100 mg of flakes of one color, or 50 mg each of red and green when both colors were used. Flakes were added to melted tallow (90% beef tallow, 10% white beeswax, 25 mg rhodamine B) in a bait mold and the tallow was allowed to harden. The 4–5 g tallow baits were then offered to coyotes in individual kennels at our research facility near Logan, Utah. Coyote care, housing, and support facilities were designed to comply with Animal Welfare Act and Denver Wildlife Research Center, Animal Care and Use Committee standards.

Before tests began, coyotes were fed about 600 g per day of commercial mink feed produced by the Fur Breeders Agricultural Co-operative Association, Logan, Utah. Kennels were cleaned daily with pressurized water just before feeding, and coyotes had water available continuously from an automatic system.

Experimental baits were fed to 16 coyotes; seven were offered baits with red and green, and three each were offered baits with red, green, or blue flakes. Scats collected from three coyotes before they were baited served as experimental controls.

Test coyotes were fasted for 1 day, then they were fed about 600 g of jackrabbit (*Lepus californicus*) or domestic sheep parts, including hair, wool, skin and bone for 3 days. After 3 days on the jackrabbit/sheep diet, one bait with colored flakes was offered to each coyote just before its fourth jackrabbit/sheep meal. For the following 5 days, newly deposited scats were collected just prior to kennel cleaning, kennels were cleaned, and coyotes were fed jackrabbit/sheep as before.

Scats from each coyote were collected and placed in paper bags individually labeled with the date, coyote and kennel number and initials of the collector, and were allowed to dry. After approximately 1–8 months, contents of each bag were examined separately, and results were recorded and analyzed using a split-plot design in analysis of variance (ANOVA) with a general linear models procedure (PROC GLM) on the SAS system (SAS Inst. Inc., 1988). When ANOVA indicated significance ($p \leq 0.05$), we compared differences among individual methods using Tukey's studentized range test (SAS Inst. Inc., 1988).

Additionally, one small scat from one coyote in each color-test group was collected daily for 3 days following baiting and was weathered for 30 days. Scats for weathering were placed on an 45.7 \times 85 cm stainless steel tray that was covered with brown paper. The surface was divided into 12

compartments (4 groups \times 3 days) separated by 1.9×2.5 cm wooden strips. Each day the scat selected was placed in a separate compartment that was labeled with the coyote number and date collected. The tray was kept outdoors, exposed to the elements in a welded wire (2.2 cm square mesh) enclosure ($45.7 \times 45.7 \times 76.2$ cm) to protect the scats from disturbance by large rodents and birds. After 30 days, scats were stored inside at room temperature until examined for flakes using the same procedures as used for non-weathered scats.

Scats were examined at $7\times$ under a variable powered, $7\text{--}30\times$, dissecting microscope with its own light source. The $7\times$ power was sufficient for flake detection and allowed the largest field of view. Examinations were made systematically following a parallel strips pattern. Forceps were used to separate and move scat material around on plates while searching for flakes; these were rinsed, wiped dry and inspected after each scat examination to ensure that colored flakes were not transferred from one sample to another. The presence of flakes and the colors found were recorded for each coyote, each day, for each examination method.

For examination, the entire surface of each scat in each bag was scrutinized for flakes. Next, all scats from the bag were crushed, spread on a white 9-in paper plate, and examined again. Finally, crushed material was washed in a nylon bag, and the bag and contents were examined again on a new paper plate.

For the washed-scat examination, bags were constructed from uncoated, bluish-white nylon cloth which was woven tightly enough to retain flakes (1.9 oz, 80 denier). The material was cut into 7×14 in pieces and cut edges were flame-sealed to prevent unraveling during washing. Unraveling could allow flakes to escape from a bag to contaminate others. Each piece was folded to 7×7 in and double seamed, using the smallest stitch possible on a sewing machine. The first seam, $1/4$ in from the edge, was sewn continuously to close two of the three unfolded edges. The third edge remained open for filling. The second seam was $1/8$ in from the edge to reinforce the first and help prevent flakes from escaping. Scat material was bagged with a label of plastic tape containing the coyote number and the collection date written in permanent ink. After labeling, bag openings were closed with several tight twists of a rubber band.

Bags were divided into three groups for washing; large, medium and small, based on the volume of scat material per bag. Each group was soaked over night to soften the scats and was washed in a clothes washing machine with a center agitator. After the washtub was full, water was run into the tub at the same rate it drained until the water changed from brown to clear. Bags were dried in an automatic clothes dryer and examined.

RESULTS

No flakes of any color were found by any examination method in scats deposited by three coyotes the day before baiting. After baiting, detection of flakes showed differences among coyotes ($F = 6.00$, $p = 0.0001$) and among detection methods ($F = 30.21$, $p = 0.0001$), but not among flake colors ($F = 0.79$, $p = 0.521$).

Among detection methods, correct colored flakes from scats of coyotes baited with all colors or color combinations were found for averages of 2.4 days (range = 1.9–3.3) in surface-examined scats, 2.3 (range = 2.0–2.7) days in crushed scats, and 3.8 days (range = 3.6–4.0) in washed scats (Table 1). Washing scats provided significantly ($F = 0.148$, 24 df, $p = 0.05$) longer detection compared to surface and crushed-scat exam-

TABLE 1
% of Correct Color or Color Combination and Average Number of Days of Detection of Colored Flakes Found in Coyote Scats by Examination of Scat Surface (S), Crushed Scats (C), and Washed Scats (W)

No. of coyotes	Examination method	Day of deposit post baiting					Average days per method ^a
		1	2	3	4	5	
% scats with red and green flakes							
7	S	86	71	29	0	0	1.9
	C	86	86	43	0	0	2.1
	W	100	100	100	29	29	3.6
% scats with red flakes							
3	S	67	100	67	0	0	2.3
	C	67	100	33	0	0	2.0
	W	100	100	100	67	33	4.0
% scats with green flakes							
3	S	100	67	100	0	0	2.7
	C	100	100	67	0	0	2.7
	W	100	100	100	33	67	4.0
% scats with blue flakes							
3	S	100	100	67	33	33	3.3
	C	67	100	67	33	0	2.7
	W	100	100	67	67	33	3.7
Total % scats with all flake colors							
16	S	88	81	56	6	6	2.4
	C	81	94	50	6	0	2.3
	W	100	100	94	44	38	3.8

^aMaximum of 5 possible days.

inations; there was no significant difference in detection between the latter two methods.

No color fading or color separation from silver bases were observed on scats after weathering and storage. Times from scat deposit to examination varied from 3–4 weeks (8-28-86–9-22-86) to about 8 months (8-29-86–4-3-87), including 30 days of outside weathering and 7 months of inside storage at room temperatures. The results indicated the high stability of the colored flakes in coyote scats.

Flakes with the wrong colors were detected in 2 of 80 (2.5%) scat-surface, in 0 of 80 (0.0%) crushed-scat, and in 7 of 80 (8.7%) washed-scat examinations. Also, in some washed scats, color separation from silver bases was observed. Possible reasons for these observations will be discussed.

DISCUSSION

The flakes were bright and easily observed with the dissecting scope, and test colors contrasted well in scats from coyotes fed jackrabbits and sheep. In some areas or at specific times of the year, based on knowledge of coyote food habits, colors that would not contrast with expected components of coyote scats might be avoided. Additionally, in over-washed or over-dried bags, or both, the color coating of flakes sometimes separated from the silver backing used as a colorant base. The problem was minor because colored portions were still present and easily detected. However, if silver flakes were used in baits, separation of colors from silver bases could be confusing, and use of silver probably should be avoided. Fall and Johns (1987) also noted that silver-colored flakes should be avoided because silver appeared as a minor contaminant in flake stocks and warned against using silver with other collars.

Wrong colors were sometimes observed and possible reasons include, coyotes eating portions of baits scattered by coyotes in adjacent kennels, color contamination in supplies purchased from the manufacturer, color contamination during bait and scat handling, or flakes escaping from original bags into wash water and catching in necks of other bags where they were observed. However, wrong colors were represented by very few flakes, sometimes only one, compared to numbers of flakes of the expected colors, hence the detection of wrong-color flakes did not necessarily compromise the examination results.

Fall and Johns (1987) observed that colored flakes were unaffected by the digestive system passage in rats. We found that these flakes were unaffected by digestion and elimination by coyotes and by scat storage for

8 months. Additionally, Fall and Johns (1987) reported that 50% of rats retained flakes 72 h (3 days) after feeding. In coyote scats with wash bag detection, reliable marking was nearly 100% on day 3 post baiting, about 50% on day 4 and about 30% on day 5.

Colored flakes are easily detected and persistent in coyote scats and would make useful short-term markers in coyote baiting investigations and perhaps in other feeding studies. Wash-bag examination provided the longest detection of methods tested and would likely be useful in detecting most kinds of inert markers.

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