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Longevity of DayGlo fluorescent particle marker used to mark birds in flight pen and field

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Abstract. Spray application of fluorescent particles is a widely used and very valuable technique for marking birds. This remains one of the few practical means to mark large numbers of birds for monitoring movement, despite recent availability of a variety of more technologically advanced options. We monitored the longevity of the DayGlo fluorescent particle marker on red-winged blackbirds under simulated field conditions in a flight pen, and in an observational field experiment. In the pen study we banded 52 red-winged blackbirds with individually numbered leg bands, and sprayed them with DayGlo fluorescent particle marker from a distance of ~50 cm, on 1 December 2004. These birds were recaptured and DayGlo fluorescent particle marks assessed 11 times until 10 August 2005. All 31 surviving birds at the conclusion of the study (i.e. after 254 days) retained at least some DayGlo fluorescent particle marker on one or more body regions. Wings retained DayGlo fluorescent particle marks longer than other body regions and thus could be used to identify marks in large-scale collections. Roosting wild blackbirds aerially marked in September and October 2005 retained marks through June 2006, 263 days after marking. The formulation used is inexpensive (US$4.00 L^{-1}), easy-to-apply at many scales and practical for many species (e.g. starlings, blackbirds, sparrows, gulls and shorebirds).

Introduction

Several external marking techniques allow determination of local and seasonal geospatial movements of birds. Techniques allowing identification of individuals after marking include numbered leg bands, neck collars for marking geese and swans (Craighead and Stockstad 1956; Samuel *et al*. 2001; Zadegan 2004; Williams *et al*. 2008), nasal markers for ducks (Sugden and Poston 1968; Lokemoen and Sharp 1985; Brook and Clark 2002), and patagial and leg tags (Cummings 1987; Claridge 1990; Linz *et al*. 1992; Carver *et al*. 1999; Houston and Bloom 2005). Neck collars and patagial tags can be read without recapture, while numbered leg bands and leg tags typically require recapture. Techniques that allow identification of marked birds out of a group include coloured leg bands and flags (Balham and Elder 1953; Hill 1992; Watt 2001; Regehr and Rodway 2003) and temporary dyes (Wadkins 1948; Moseley and Mueller 1975; Wendeln *et al*. 1996; Donehower and Bird 2005). Through single or multiple bird captures, these marking methods enable the investigation of movements of marked individuals.

DayGlo[1] (DayGlo Colour Corp., Cleveland, Ohio) fluorescent particle formulation (A/AX Pigments) (hereafter referred to as 'marker') is available worldwide for marking wild birds (Jaeger *et al*. 1986; Knittle *et al*. 1996). It is a liquid formulation that dries and adheres to the feather surface with some (material) lodging in the feather barbules (Otis *et al*. 1986). Most reported uses of this marker have been aerial applications with a fixed-wing airplane for large aggregates of birds. Recent advancements enable small flocks to be marked using a ground spray system (P. T. Oesterle, National Wildlife Research Center, Fort Collins, CO, USA, unpubl. data), and a tower spray system adapted from Bullard (1990).

Knittle *et al*. (1987) marked 10.6 million red-winged blackbirds (*Agelaius phoeniceus*) in Missouri and South Dakota, USA, with marker. Linz *et al*. (1991) and Homan *et al*. (2004) used a different marker formulation to mark 300 000 and 370 000 blackbirds in North Dakota and South Dakota, USA, respectively. After marking, birds in these studies were systematically shot and collected along migrational pathways or in breeding areas and examined for marker. Collections enabled researchers to determine turnover rates at summer roosts, and spring migration patterns to breeding sites. This technique is also useful to ascertain local movement patterns. For example, ~70 European starlings (*Sturnus vulgaris*) were marked using a remotely activated ground spray device (P. T. Oesterle, National Wildlife Research Center, Fort Collins, CO, USA, unpubl. data). Lethal collection and examination of birds at surrounding feedlots allowed researchers in this study to study the potential for disease transfer between feed lots.

This technique is one of the few practical means of marking large numbers of birds for monitoring movement. Despite its use in the field setting, only minor anecdotal and empirical data have been collected to evaluate longevity of DayGlo fluorescent particle marker on birds. Information on the longevity of the marker is crucial for determining detection capabilities. Bruggers

[1]Use of brand names does not imply USDA endorsement.

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and Bortoli (1979) documented the longevity of a variety of formulations on the wings of red-billed quelea (*Quelea quelea*) and other weaver birds (*Ploceus* spp.) for up to 115 days. Jaeger *et al*. (1986) recovered marked wings 67–100 days after marking. Knittle and Johns (1986) described a 29% pigment loss after 42 days. In contrast to the adhesive used in these earlier studies (No. 2 diesel fuel), we used Carboset 514H (Lubrizol Advanced Materials, Inc., Cleveland, Ohio, USA) as an adhesive in our formulation due to environmental concerns of spraying diesel fuel in wetlands. We are not aware of any published data describing the retention of the current DayGlo Fluorescent particle marker formulation on feathers (Johns *et al*. 1989). Thus, our aim was to monitor the longevity of the DayGlo Fluorescent particle marker on red-winged blackbirds under simulated field conditions. Additional data on longevity of marker were obtained through collections of blackbirds from a field marker study in south-eastern Missouri, USA.

Materials and methods

Flight pen study

We conducted this study at the Outdoor Animal Research Facility of the United States Department of Agriculture (USDA), National Wildlife Research Center (NWRC), in Fort Collins, Colorado, USA. All birds had been field-caught several months before the experiment, and were maintained in $4.9 \times 2.4 \times 2.4$ m cages within an open-sided building exposed to ambient environmental conditions. After marking, we placed birds into a 0.07-ha flight pen with a pole-supported net roof ($1'' \times 1''$ mesh) 7.6 m high at the centre and 2.4 m high at the sides. We provided birds free access to water, grit, and maintenance feed (2 millet : 1 milo : 1 safflower : 1 sunflower). Two wooden shelters ($1.8 \text{ m} \times 2.4$ m), three 1.5-m potted ponderosa pine (*Pinus ponderosa*) trees and white cedar (*Thuja occidentalis*) branches were available for roosting. We followed animal care criteria outlined by the animal welfare act and the NWRC institutional animal care and use committee (USA Federal Permit No. MB019065-14 and Colorado State Permit No. 04-TR060).

We used the DayGlo Fluorescent particle marker formulation described by Linz *et al*. (1991), Knittle *et al*. (1996), and Homan *et al*. (2004). A 950 mL solution of this formulation was mixed within 30 min of application. On 1 December 2004, 52 male red-winged blackbirds were banded with individually numbered leg bands and then held by the wrists and evenly sprayed on both dorsal and ventral surfaces from ~50 cm with a 1-L adjustable spray bottle. This application of marker is similar to well marked portions of birds from field application with fixed-wing airplane, and the tower spray method. We placed sprayed birds in an indoor pen ($2.1 \text{ m} \times 2.4 \text{ m} \times 2.1$ m) lined with a soft mesh net for holding while all 52 blackbirds were marked. The marker material dried in less than 1 min, and all 52 birds were then transported to the flight pen. Birds in the flight pen were exposed to direct sunlight, ambient temperatures, rain, and snow, and potential wear factors such as bathing, dusting and abrasion from vegetation and netting.

We used a modified crow-trap (Gadd 1996) to recapture birds at least monthly from December 2004 through August 2005, when birds began to moult. We tried to capture more than 75% of the birds during each assessment. Birds were placed in a burlap sack for transport to minimise human-caused abrasion of the marker. We used a model B-100A Black-Ray long-wave (360 nm) ultraviolet light in a darkened observation room to detect the marker and determine marker retention. Although marker can be visible to the naked eye, a darkened room was used to minimise error in judging the marker retention. We categorised four general body regions of the birds using the major feather tracts: head region or capital tract; body region or spinal ventral, femoral and crural tracts; tail region or caudal tract; and wing region or humeral and alar tracts. On each region we used the following categories to rate the area covered by the marker: (0) no marks visible; (1) 1–5 marks visible; (2) >5 marks visible but less than 50% of the region covered; (3) >50% of body region covered, but less than full coverage of region; and (4) marks on entire region. A mark indicated a visible spot of marker material. After the initial spray each bird was determined via visual observation to be a Category 4 for each body region.

We documented the time until marks were lost from the four regions of each bird based on recaptures by individual band numbers through the beginning of moult in August 2005. The time in day(s) until the marks of each bird reached the respective categories (i.e. Category 3, 2, or 1) was analysed non-parametrically using Kaplan–Meier (Kaplan and Meier 1958) survival analyses (up to a total of 254 days). Wilcoxon comparisons of survival curves were used to compare regions of each bird for their longevity within marking categories (Kalbfleish and Prentice 1980). Analyses were conducted using SAS PROC LIFETEST (SAS Institute 2004). Birds dying during the study were considered censored at the time of the event and were eliminated from further analyses. The analyses enabled the use of mark survival times through the censoring event.

Field study

Three mixed flocks of blackbirds (red-winged blackbirds, brown-headed cowbirds (*Molothrus ater*), and common grackles (*Quiscalus quiscula*)) estimated at 700 000, 750 000, and 2.2 million were each aerially marked once between 29 September and 2 October 2005 (post-moult) in south-eastern Missouri. We collected birds from October 2005 through June 2006 at varying intervals in the area surrounding the spray sites (Table 1). We used a model B-100A Black-Ray long-wave (360 nm) ultraviolet light in available darkened observation areas (i.e. warehouse space) to detect birds with marker.

Results

Flight pen study

We recaptured red-winged blackbirds and assessed DayGlo fluorescent particle marks 11 times between 1 December 2004 and 10 August 2005, or an average of every 23 days (s.e. $\pm$ 8.6 days). We recaptured 27–86% of the birds during each assessment period (Fig. 1). We observed 21 bird mortalities during the study. Mortalities were generally associated with recapture and handling (0–2 per recapture event). Five mortalities were definitively attributed to hawk or owl

Table 1. Summary data for mixed flocks of blackbirds collected between October 2005 and November 2006 in south-eastern Missouri, USA. Species included are red-winged blackbirds (*Agelaius phoeniceus*), brown-headed cowbirds (*Molothrus ater*) and common grackles (*Quiscalus quiscula*)

Days after marking	No. of birds collected	No. of birds marked	Percentage of birds marked
1–20	594	524	88.2[A]
21–40	261	36	13.80
41–60	306	22	7.20
61–80	737	53	7.20
81–100	135	9	6.70
101–120	99	6	6.10
121–140	33	1	3.00
141–160	105	4	3.80
161–180	0	0	0.00
181–200	0	0	0.00
201–220	211	81	38.40
221–240	110	18	16.40
241–260	8	1	12.50
260+	27	3	11.10
Total	2626	758	28.90

[A]Birds collected on day immediately following aerial spray.

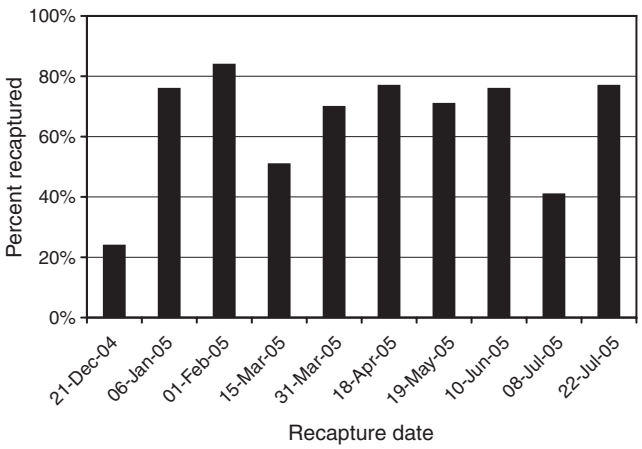

Fig. 1. Proportion of the study population (n = 52 red-winged blackbirds (*Agelaius phoeniceus*)) recaptured for visual inspection of the DayGlo fluorescent particle marks standardised to include mortality occurring between observations from December 2004 through August 2005 at the National Wildlife Research Center (NWRC) Outdoor Animal Research Facility flight pen.

predation (i.e. decapitation, talon marks observed). Predators were able to capture birds through the flight pen netting.

Median longevities for body region classification as Marking Category 4 on the body, head, tail, and wing regions were 19, 84, 77 and 154 days, respectively. Those for Marking Category 3 or better on the body, head, tail, and wing regions were 130, 133, 154 and 243 days, respectively. Strong differences were observed between different body regions for longevity within Category 4 (Fig. 2) and also Category 3 (Fig. 3) (Wilcoxon comparison of Kaplan–Meier survival curves: $\chi^2 = 144.47$, d.f. = 1, $P < 0.0001$; $\chi^2 = 70.97$, d.f. = 1, $P < 0.0001$, respectively).

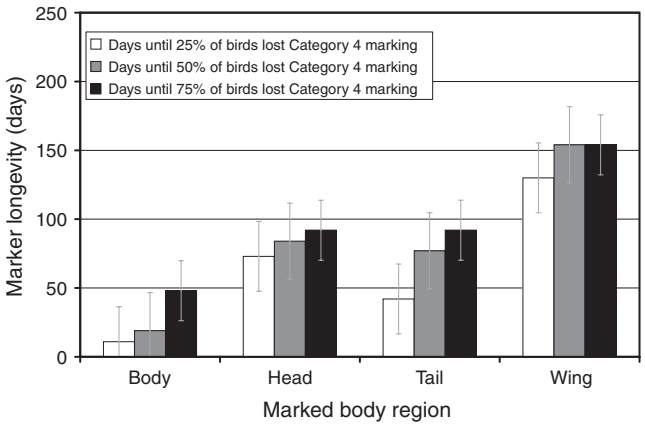

Fig. 2. Longevity of Category 4 (i.e. marks on entire region) DayGlo fluorescent particle marker among marked red-winged blackbirds (*Agelaius phoeniceus*). Error bars represent standard error.

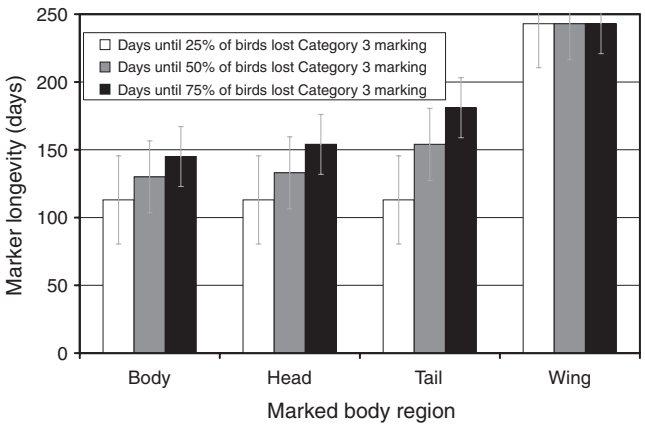

Fig. 3. Longevity of Category 3 (i.e. >50% of body region covered, but less than full coverage of region) DayGlo fluorescent particle marker among 25%, 50%, and 75% of marked red-winged blackbirds (*Agelaius phoeniceus*). Error bars represent standard error.

Field study

Birds were shot during 27 collections between October 2005 and June 2006 or an average of every 11 days in the area surrounding the spray sites. In total, 2626 birds were collected. We had no means of knowing how many of the 2626 had been sprayed, but 758 of the collected birds were found to have marks. Three of the 758 birds with marks were collected on 21 June 2006, or 263 days after marking, indicating the potential for long-term mark retention during the period between moults (Table 1).

Discussion

All test birds remaining at the conclusion of the pen study retained at least some DayGlo fluorescent particle marker through 254 days following our simulated aerial application of the marker. Moult precluded further longevity testing. All birds still had regions with Category 2 markings (i.e. more than 5 marks visible but less than 50% total region covered) at the conclusion of the study. The level of initial spray of each bird in

the flight pen study is supported by the results of the field study, which found one bird with fluorescent particle marks at 259 days after marking and three birds with fluorescent particle marks at 263 days after marking.

The DayGlo fluorescent particle marks on the wings (both dorsal and ventral sides) lasted 243 days as Category 3, more than 89 days longer than those on the head, tail, or body. The feathers of the wing have pennaceous barbules that generally are larger and have more highly developed barbicels than those of body contour feathers (Stettenheim 1972), and thus are able to retain the DayGlo fluorescent particle marks longer. Although tail feathers are of the same type as wing feathers and so have the same highly developed barbicels, they are used for balance and therefore are exposed to greater abrasion due to perching, and thus do not retain DayGlo fluorescent particle marks as long as do wing feathers.

Precautions were taken during the multiple recaptures to minimise marker wear due to handling to ensure that marker wear was due to bird behaviour not human handling. Blackbirds were transported in burlap bags instead of hard plastic transport boxes. Even though these precautions were taken, flecks of the marker were still present in the burlap bags after each assessment. During the study several birds became accustomed to the modified crow trap and entered and exited the trap before we could examine them for DayGlo fluorescent particle marks. These birds, although captured fewer times overall, showed marker longevity similar to that of birds captured during each recapture, indicating that the recapture method did not cause undue degradation of the marker.

Toxicity testing of the DayGlo particle formulation at concentrations ranging from 25 to 6000 ppm on four species of fish showed minimal mortality to two species (rainbow trout (*Salmo gardneri*) and bluegills (*Lepomis promelas*)) at concentrations of 6000 ppm. During actual marker-spray operations, water concentrations typically do not exceed an average of 5 ppm (Bills and Knittle 1986). In a study to simulate contamination of upland habitat, egg production, fertility, bodyweight and food consumption were not negatively impacted in common quail (*Coturnix coturnix*) that ingested feed treated with 6.12 mL lb^{-1} of DayGlo particle formulation (Scott *et al*. 1984). Thus, the levels that we applied in our study would not be a cause of mortality to our study birds.

Application of DayGlo fluorescent particle marker formulation by spraying is a widely used and very valuable technique for marking birds. Despite the high-tech options available, this remains one of the few practical means to mark large numbers of birds for monitoring movement. Probably, this is the only economically feasible method for such applications. Despite the method's wide use, this is the first designed study to evaluate marker longevity. The current cost of this formulation is approximately US$4.00 L^{-1}. This technique of mark and collection would be cost effective at many scales (for example we previously marked 2 million blackbirds with only 379 L of the formulation: Cummings *et al.* unpubl. data) and with many species of birds that congregate either in local small flocks or in larger aggregates including starlings, blackbirds, sparrows, gulls and shorebirds.

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