A New Method to Dye-Mark Wild Birds

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ABSTRACT

Dye-marking of Great Egrets (Ardea alba) *and Great Blue Herons* (Ardea herodias) *was conducted in an intertidal salt marsh in South Carolina, as part of a study on wader energetics and bird/dolphin interactions. We used remotely triggered Spraymore paintball land mines to apply dye. These compressed gas-powered mines were portable, re-useable, and capable of spraying liquid dye. Using an inkjet printer ink-based dye, we were able to mark and re-identify one Great Blue Heron and one Great Egret successfully. No long-term behavioral changes were apparent in marked birds, which were re-sighted in the same areas and engaged in normal behaviors following the application of dye. Dye markings lasted as long as 44 days. The use of Spraymore paint mines is a viable option for marking any birds that return regularly to specific observable areas, particularly when birds in these areas are not marked or captured easily using traditional methods.*

INTRODUCTION

The ability to differentiate between individual animals is important for many scientific studies. For bird research, leg bands are the traditional long-term method of labeling an individual (Marion and Shamis 1977). For many studies, shorter-term, individual markers may be more appropriate. Color-marking of birds is an accepted method of differentiating between individuals over shorter time frames, depending on the specifics of the method employed.

Many marking techniques, including banding, require capture and handling of wild birds, as well as the ability to physically access the environment in which netting will occur. Physical access to some environments can be quite difficult; for instance, sticky, unstable "pluff" mud in salt marshes greatly restricts movement of researchers and equipment through the marsh, making it difficult to set up mist nets or to access roosting birds, and there is little cover for researchers hoping to evade detection by study animals. Additionally, human incursions may be greatly disruptive to the environment itself. Other environments, such as dense vegetation or shifting sand, may have similar constraints. Capture and handling of birds causes them stress, and may have adverse effects on bird health and survival. Leberman and Stern (1977) describe short-term weight loss in songbirds caused by handling stress, and Green (1978) notes the potential for leg paralysis and wing strain in captured waders. The handling of some large birds, including those with sharp talons or bills also poses some danger to researchers, who may wish to avoid handling such animals, if possible.

In lieu of capturing and banding birds, several methods have been described for color-marking wild birds. In their review of bird-marking techniques, Marion and Shamis (1977) list a number of studies in which captured birds were marked with color, or in which colored feathers were attached to captured birds. Capture-free methods also exist for dye-marking. Belant and Seamans (1993) and Donehower and Bird (2005) applied paste dyes to perches, nests or eggs, so that returning birds would smear dye on their feathers. Moffitt (1942) described the use of "bombs" filled with dye, which could be thrown near birds, splashing them with dye, a method that may work best when the observer can be well-hidden and escape routes for the birds are limited. Rodgers (1986) and Waugh (1998) used a pressurized sprayer

to mark resting birds, a method which requires knowledge of and access to roosting places. Moffitt (1942) also used a remotely triggered pressurized sprayer hidden in an area frequented by his target species. This device was essentially a pressurized tank of dye buried in a lek area, with a valve that was opened by pulling on a string. Wendeln et al. (1996) devised a more complicated sprayer device, designed to be left in the field and automatically triggered when a bird roosted on it. These devices are not necessarily well suited for inaccessible environments or for all species of bird and may require substantial setup.

Several different types of dyes have been used to mark birds in the past. These include aniline dyes and leather dyes, human hair dye and batik dye (Donehower and Bird 2005; Rodgers 1986; Ellis and Ellis 1975; Moffitt 1942), Rhodamine B and picric acid (Waugh 1998; Wendeln et al. 1996; Belant and Seamans 1993; Paton and Pank 1986), silver nitrate (Wendeln et al. 1996) and malachite green (Belant and Seamans 1993). Many of these dyes may be mixed into a paste with petroleum or silica gel or propylene glycol, while others are mixed with isopropyl alcohol to better penetrate feathers. Some of these dyes may pose hazards to both researchers and marked birds–picric acid can form explosive crystals and Rhodamine B may be a carcinogen (Gaunt and Oring 1997; Donehower and Bird 2005).

At varying concentrations, these dyes last from four weeks to several months, with some remaining visible until marked feathers were molted. Rodgers (1986) reported no behavioral changes in marked nestlings, although he recommended against marking the mouth, nostrils and eyes. Wendeln et al. (1996) also observed no negative effects of dye on bird health, and Moffitt (1942) reported some temporary discomfort to marked birds, but that normal behavior resumed within a day. Gaunt and Oring (1997) note that dye may reduce feathers' ability to insulate in cold or wet weather. Ellis and Ellis (1975) point out the potential dangers of changing color markings of birds, especially when color markings mimic sex or age signals. When the proper precautions are taken to avoid negative effects on bird health or behavior, dye marking can be an effective marking technique for short-term studies.

What is needed, then, is a simple, effective way of marking wild birds that requires no handling, no access to the birds' roosts and simple deployment of equipment. Safe marking of birds also requires a simple, non-toxic dye with lasting visibility. As part of a study on wader energetics and bird-dolphin interactions in a South Carolina salt marsh, (Fox and Young, 2012), we sought to develop a device to meet this need. Individual herons and egrets are difficult to tell apart, as they generally lack individual-specific markings. Their roosting places are inaccessible, and their salt marsh foraging grounds, where this study occurred, are characterized by tidal creeks, pluff mud, and oyster reefs, inaccessible terrain for most capture techniques. Our device needed to be portable, simple to set up in the intertidal salt marsh, and able to effectively deliver dye at the appropriate time. The devices used in this study were developed from commercially available *Spraymore* paintball land mines. *Spraymore* mines are re-useable, powered by any compressed gas, can spray any liquid dye, and are controlled by the researcher so that they can be remotely "detonated" by the researcher with an electronic trigger at the correct moment. Using these mines and a dye developed for this study, we were able to successfully mark and re-identify Great Blue Herons *(Ardea herodias)* and Great Egrets *(Ardea alba)* in a South Carolina salt marsh.

METHODS

Our study site was located in the salt marshes of Bull Creek, in the Calibogue Sound estuary in Beaufort County, SC. Bird-marking occurred in Jun-Sep 2009 and May-Jul 2010. Research was conducted from a 5.5 m aluminum boat, as the marsh was not passable on foot.

Initial attempts to mark birds during the summer of 2009 utilized air-pressurized squirt guns (Buzz Bee Toys, Item # 32700) filled with aniline dye mixed with water or with water and ethanol. Compared to water, the squirt guns were able to spray the dye with no apparent reduction in range or power. However, range was still extremely limited (generally less than

6 m, and further reduced by wind). Because of this limited range, all birds flushed before they could be sprayed with dye. Birds appeared to associate the boat with dye-marking attempts and were even more wary of subsequent attempts to spray them. Tests also indicated that aniline dyes, even when mixed with alcohol, did not effectively penetrate feathers and could be washed off easily.

Dye-marking was attempted again in summer 2010 with Spraymore Mega paintball land mines (http://www.paintballlandmine.com) [Fig. 1]. These mines used compressed gas (either air or CO_2) to spray a liquid, and were triggered by an electronic remote control, which was effective at distances of over 100 m (line of sight). These mines were marketed as "water resistant," but with straightforward modifications, we were able to waterproof the electronic components completely for use in the intertidal salt marsh. The spray radius of these mines was 2 -5 m, depending on gas pressure, and was reduced severely by wind. Mines could be refilled with both dye and compressed gas, and each held approximately 0.6 liter of liquid dye.

Dye consisted of approximately 25 milliliter of color inkjet printer ink (Jet Tec Color Refill Kit) diluted in approximately 950 milliliter isopropyl alcohol and 950 milliliter of water. The inkjet printer ink came from an ink cartridge refill kit purchased at an office supply store. For lighter colored inks, ink concentration was increased in the dye.

The mines were placed in the intertidal salt marsh in areas where birds were known to congregate as they followed dolphins. Mines were also placed near foraging birds in the marsh, in hopes that their hunting would lead them to walk into the spray zone. Boat-based researchers triggered the mines when a bird appeared to be within range. Mines could be easily placed and retrieved and no accidental sprayings occurred.

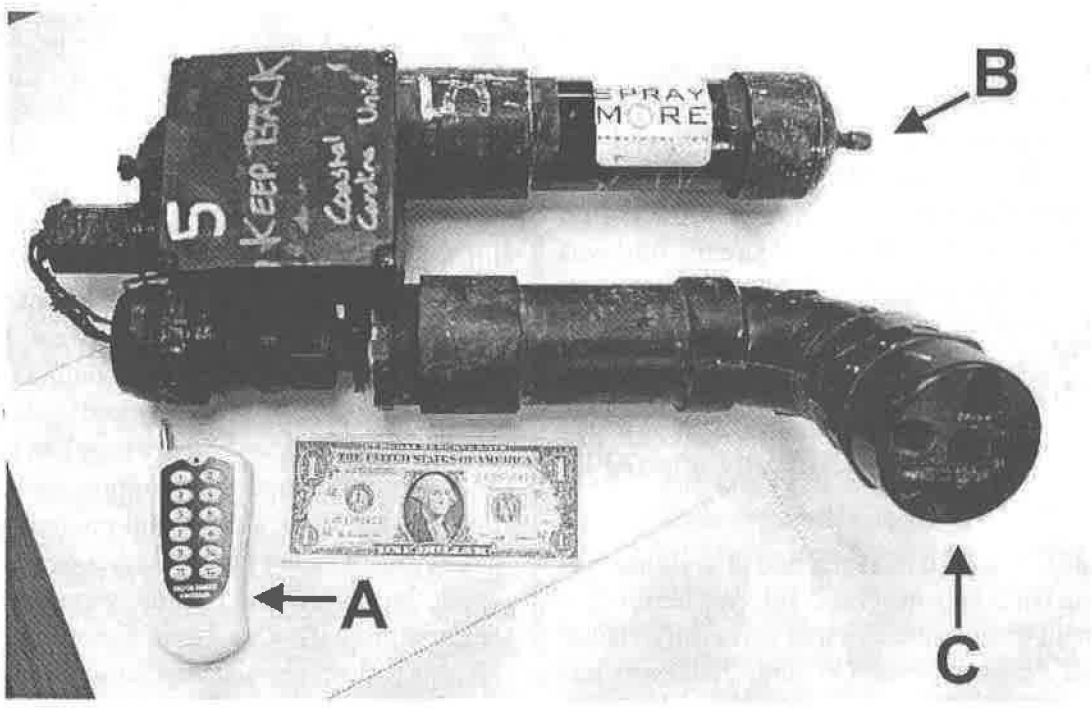

Fig. 1. A *Spraymore Mega* paintball land mine (with modifications for waterproofing). A: multi-channel electronic trigger; B: Schrader valve for filling with pressurized gas; C: Spray nozzle.

RESULTS

Marking efforts with *Spraymore* paintball land mines and subsequent observation surveys were conducted between 6 May and 26 Jul 2010. Over a dozen attempts were made to spray dye on target species. When a mine was triggered, it produced a visible spray and hissing sound as gas escaped rapidly, but no startling bang or pop. These cues generally caused unsprayed birds in the immediate vicinity to flush. However, startled birds usually circled around, landed, and resumed foraging in the same area. During this study, four birds were marked, two of which were re-sighted. All marked birds did take evasive action and left the area after being sprayed with dye.

On 15 May 2010, we successfully marked one Great Egret. The undersides of the wings and tail were sprayed with a purple dye as the bird walked along the muddy creek bank. These markings were only partially visible when the bird was on the ground, but clearly visible during flight. The egret flushed when sprayed and was not observed again on 15 May, but was re-sighted in the same general area on 16 May, and was subsequently spotted in the same area throughout May and Jun 2010, until the last observation on 28 Jun 2010 (44 days after being tagged). Over this time, dye markings did visibly fade. This bird had been associated with dolphins several minutes before being marked and was marked while foraging. Re-sightings all occurred within approximately 800 m of where the bird was marked, along one particular tidal creek, although one observation ended when the bird flew south across a major tidal creek. Post-marking observations included foraging behavior and continued association with dolphins, indicating that the bird's behavioral patterns were not greatly affected by being marked.

The second successful marking and re-sighting was of a Great Blue Heron. On 9 Jul, the heron was sprayed with bright blue dye that was easily visible on the bird's white throat. This individual was last observed on 11 Jul. On all three days (including 9 Jul before it was marked), the heron was foraging in associating with dolphins. All re-sightings were within approximately 500 m of where the bird was marked.

Two additional Great Egrets were marked but not re-sighted. These birds were only lightly dyed on the underside of the wings, markings which would have been invisible unless the birds were in flight. The light coloration would have made re-sighting these birds difficult even in the most ideal circumstances.

DISCUSSION

Using *Spraymore* paint mines, we were able to mark four large wading birds in a salt marsh habitat, of which two were re-sighted. These marked individuals helped document individual foraging behaviors and movement. The dye mixture used in this study was effective at coloring feathers and was visible for as long as 44 days on the white plumage of a Great Egret. These birds were marked while they foraged along muddy salt marsh creek banks, a habitat that would have made capturing the birds extremely difficult.

This method of dye-marking appeared to have no lasting negative impacts on marked birds. The *Spraymore* paint mines were quiet enough that nearby birds were only momentarily flushed and, while marked birds left the area immediately after being sprayed, the dye did not cause any apparent longer-term changes in a marked bird's behavior. We feel that the possibility of damage to sensitive areas of target birds, specifically the eyes, was minimal because marked birds had time to react to the mine detonation so that their wings were typically up when hit. Presumably this would allow them more than enough time to blink, if necessary. Both the marked Great Egret and the marked Great Blue Heron returned to the same area and engaged in the same behavior after being dyed. Marked birds continued to forage in the same area where they had been sprayed and even continued their unique association with bottlenose dolphins. They did not demonstrate any avoidance behavior towards other paint mines on the creek banks. Marked birds were also observed associating with other Great Egrets and Great Blue Herons with no unusual interactions between marked and unmarked birds. However, this study found evidence of extended survival by only one of four marked birds, so additional tests on long-term effects may be advisable.

Dye markings on one Great Egret were visible for 44 days and, by increasing the ratio of dye to water/ isopropanol mixture, it is likely that markings would have lasted even longer. The dye did fade slightly over time, especially on tail feathers that occasionally dragged in the water.

In this study, only two dyed birds were re-sighted. This may be due to the location of the birds relative to the mines. Tall birds walking (or gliding) over mines placed on the ground were sprayed mainly on the undersides of their bodies and wings. It may be difficult to spot a lightly dyed bird, particularly if the dyed feathers are on the underside of the wing. The Great Blue Heron was seen for only three days after being dyed. It is possible that the disappearance of this heron indicated injury or death, but it seems unlikely that negative impacts were caused by dye-marking, as this bird appeared undamaged and behaviorally unaffected for two days subsequent to marking. Great Blue Herons were less common than Great Egrets over the course of our study on wading bird-dolphin interactions, and it seems more likely that this bird simply left the study area.

The use of remotely triggered *Spraymore* paint mines to mark wild birds is a viable option for any birds that regularly return to specific observable areas, particularly when birds in these areas are not easily marked or captured using traditional methods. Birds with regular foraging grounds, nesting birds or birds at a lek would all be suitable targets for this method.

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