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EVALUATION OF DYES AND TECHNIQUES TO COLOR-MARK INCUBATING HERRING GULLS

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Abstract.—The effects of three fixatives (acetic acid, isopropyl alcohol, propylene glycol) and carriers (petroleum jelly, vegetable shortening, an oil-based silica gel) for improving retention of Rhodamine B (RB), Malachite Green (MG) and Picric Acid (PA) dyes by feathers were evaluated to develop a mixture suitable for marking incubating Herring Gulls (*Larus argentatus*) via application to their eggs. Isopropyl alcohol improved feather retention of RB and PA significantly more than propylene glycol. Propylene glycol improved retention of MG significantly more than isopropyl alcohol. Overall, silica gel-based dye mixtures had significantly greater retention by feathers than petroleum jelly-based mixtures. Color-marks on gulls with RB and silica gel were also more visible with greater retention than marks on gulls with RB and petroleum jelly. Application of the carriers to domestic chicken eggs during day 1 or 11 of incubation, however, caused 100% embryonic mortality. Hatch success of gull clutches was also significantly reduced by direct application of dye to gull eggs or to dummy eggs placed in nests for 24 h. To improve longevity of color marks via application of dyes to eggs, use of an oil-based silica gel as the dye carrier is recommended, as is use of a dummy egg or marking only 1 egg of a clutch late in incubation to reduce mortality. To improve dye retention via topical application, solutions of 35% isopropyl alcohol with RB and PA and 99% propylene glycol with MG are recommended.

EVALUACIÓN DE TINTES Y TÉCNICAS PARA MARCAR INDIVIDUOS DE *LARUS ARGENTATUS* DURANTE EL PERÍODO DE INCUBACIÓN

Sinopsis.—Se evaluó el efecto de tres fijadores (acido acético, alcohol isopropílico y glicol de propileno) en portadores (vaselina, manteca vegetal y aceite en gelatina de sílice) para mejorar la retención de tintes en las plumas tales como de rodamina B (RB), verde de malaquita (VM) y ácido pícrico (AP). El objetivo del trabajo lo fue el desarrollar una mezcla apropiada que se pudiera aplicar a los huevos para marcar a individuos de la gaviota *Larus argentatus* durante el período de incubacion. El alcohol isopropílico mejoró significativamente la retención en las plumas de RB y AP sobre el glicol de propileno. Sin embargo el último mejoró significativamente la retención de VM que el alcohol isopropílico. En general, la retención de mezclas de tinte y gelatina de sílice fue significativamente mayor que las mezclas en las cuales se utilizó vaselina. La tinción de plumas con la mezcla de gelatina y RB no tan sólo se retuvo por mayor tiempo sino que resultaron más visible que las mezclas de vaselina con RB. La aplicación de los portadores a huevos de pollo durante los días 1 o 11 de incubación, causó un 100% de mortalidad. El éxito de eclosionamiento de las gaviotas se redujo significativamente como consecuencia de la aplicación directa de los tintes a huevos de estas aves o a la colocación de un huevo artificial teñido, por un período de 24 h. Para incrementar la durabilidad de tintes aplicados a través de los huevos, se recomienda el uso de gelatina de sílice en una base de aceite como portador, aplicado a un huevo artificial o a uno natural de la camada en etapas tardías del período de incubacion, para de esta manera reducir la mortalidad embrionaria. Para incrementar la retención de tintes aplicados directamente a las plumas se recomienda una solución de alcohol isopropílico al 35% con RB o AP, o una de glicol de propileno al 99% con VM.

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Considerable effort has been devoted to the use of dyes for color-marking many species of birds (see reviews by Day et al. 1980, Marion and Shamis 1977). Although several investigators have used fixatives in an attempt to increase retention of dyes by feathers and to improve visibility (Kozlik et al. 1959, Moffitt 1942, Wadkins 1948), their effects have not been quantified.

In an effort to determine adult Herring Gull (*Larus argentatus*) movements and use of landfills in northern Ohio, we color-marked 1392 three-egg gull clutches during 1991 using a technique similar to that of Paton and Pank (1986). Although not quantified, the number of three-egg gull clutches with ≥1 hatched chick appeared considerably lower for marked vs. unmarked nests. Paton and Pank (1986) did not observe any mortality of Cattle Egret (*Bubulcus ibis*) eggs; however, they recommended that further research be conducted to assess hatch success adequately. Cavanagh et al. (1992) marked incubating gulls (*Larus* spp.) using dummy eggs, but did not provide data on hatch success. Paton and Pank (1986) were able to identify marked cattle egrets for 2–6 mo at distances ≤200 m; whereas Cavanagh et al. (1992) were able to identify marked gulls for ≥4 wk. Our initial observations suggested that marked Herring Gulls could not be identified with confidence about 4 wk after marking. Limited retention of a dye by any species reduces its effectiveness as a marker. Also, mortality of eggs would modify behavior of marked adults. Evaluation of different dyes, fixatives and carriers under controlled conditions should provide information useful for the development of more visible markers with improved retention and minimal embryonic mortality.

Our objectives were to: (1) assess the effects of petroleum jelly, vegetable shortening and an oil-based silica gel as dye carriers on egg viability, (2) to evaluate the retention and visibility of Rhodamine B (RB), Malachite Green (MG) and Picric Acid (PA) dyes mixed with various fixative and carrier combinations by feathers, and (3) to develop a color marker for incubating Herring Gulls that could be applied to their eggs with minimal embryonic mortality and would be readily visible during field observations from the time of application until the feathers molted.

METHODS

Laboratory Trials

Application of carriers to eggs.—We obtained brown domestic chicken (*Gallus gallus*) eggs from a local commercial producer. All eggs were handled using rubber gloves. For petroleum jelly and vegetable shortening, each group of 40 eggs was weighed, ranked by weight, and temporarily divided into eight subgroups of five eggs. One egg from each subgroup was randomly assigned as a control or one of four treatments; thus, each treatment was applied to eight eggs. Each subgroup received petroleum jelly or vegetable shortening applied during day 1 or 11 of incubation. We applied 0.5 ml of carrier to each designated egg using a 3-ml syringe without a needle. We coated eggs entirely using a sponge brush because

our field observations indicated that incubating herring gulls spread dye mixture over eggs entirely. We distributed eggs randomly within an incubator (40 egg capacity) with automatic turners (GQF Mfg., Inc., Savannah, Georgia). Eggs were maintained according to manufacturer's specifications. We also evaluated an oil-based silica gel, prepared by mixing 50 ml of heavy mineral oil (viscosity = 162) with 6 g of amorphous fumed silica (TS-530; Cabot Corp., Tuscola, Illinois). The silica gel evaluation was independent of, but similar to, that of the other carriers. The only difference was the use of 39 eggs, similarly divided into three groups of 13 eggs each. The treatments were silica gel applied during day 1 or 11, and a control. We conducted five incubator trials using petroleum jelly and vegetable shortening and one incubator trial using the silica gel.

At day 25 of incubation, all unhatched eggs were opened to assess embryonic development (Ohio Cooperative Extension Service 1990). Eggs with embryos that had developed to at least day 19 of incubation were considered hatched. Excluding eggs that received a treatment on day 1, all infertile eggs were excluded from analyses.

We used randomized block General Linear Models procedure (GLM; SAS Institute, Inc. 1988) to determine the effects of petroleum jelly and vegetable shortening treatments on egg viability. Each group of eggs within an incubator was used as a block. If significant differences ($P < 0.05$) occurred, Tukey tests were used to examine which treatments differed.

Dye/fixative solutions.—For trial 1, RB was mixed with the following 60-ml solutions: (1) 6 g RB with water, (2) 3 g RB with water, (3) 3 g RB with 70% isopropyl alcohol, (4) 3 g RB with 70% isopropyl alcohol and 2% glacial acetic acid, (5) 3 g RB with 35% isopropyl alcohol and 2% glacial acetic acid and (6) 3 g RB with 2% glacial acetic acid. For trials 2–4, 3 g of RB, MG and PA were each mixed with 60 ml of 30%, 70% and >99% isopropyl alcohol or 30%, 70%, and 99% propylene glycol. Water was used as the diluent for all solutions. As the toxicological properties of these dyes are not fully understood, they should be handled with caution.

White breast and abdominal feathers (rachises about 40–45 mm in length) were collected from captive domestic ducks (*Anas platyrynchos*). Feathers were attached individually to 3d or 4d finishing nails by tying the feather shaft to the nail head using cotton thread and applying a small amount of glue to the tied area.

For each trial, 60 feathers were divided randomly into six groups of 10 feathers; each group was randomly assigned a treatment (solution). Feathers were treated by inverting them two at a time into a solution and stirring continuously for 10 s. Feathers were then dried with a hair dryer using cool air. After drying, each feather was rinsed in 0.5 l of cold tap water for 30 s while stirring continuously. Feathers were air dried after placing them in a 6 × 10 grid made by drilling holes in a board at 5-cm intervals. The 10 feathers of each treatment were placed randomly in one of the 10-hole rows.

Feathers were randomly assigned a new position in each six-hole row that contained one feather of each treatment, such that 10 series were created. For each trial, five or six observers visually ranked each series of six feathers in descending order of the amount (intensity) of dye present. After ranking, feathers were replaced in their original positions, individually rinsed in 0.5 l of water, and air dried overnight. The following day, we assigned new random positions for feathers within each six-hole row, which were ranked a second time by the same observers.

Dye/fixative/carrier mixtures.—Based on the previous evaluation, we mixed six solutions, two each using 1 g of a particular dye and 2 ml of the most effective fixative. The two solutions of each dye/fixative combination were mixed with either 20 g of petroleum jelly or 20 g of an oil-based silica gel. We also mixed 1 g RB with 20 g of petroleum jelly as we had used during 1991 field work for comparison with other RB mixtures. Thus, seven dye/fixative/carrier treatments were made (3, 2 and 2 treatments of RB, MA and PA, respectively).

Seventy feathers were assigned randomly to seven groups of 10 feathers; each group was randomly assigned a treatment. We saturated individual feathers by completely coating each with about 1 ml of one treatment by hand while wearing rubber gloves. To simulate preening, excess material was removed from each feather by passing it three times through a paper towel held between an index finger and thumb. Feathers were then air dried overnight.

After drying, each feather was rinsed for 15 s with continuous stirring in 0.25 l of cold tap water. As on the previous day, additional treatment material was removed from each feather. Feathers were placed randomly in a board with a 7 × 10 grid so all feathers of a particular treatment were in a 10-hole row. Feathers were maintained indoors with exposure to sunlight and the board was turned 180° daily to expose all feathers equally. This procedure was repeated 5 d per week for 8 wk.

In each seven-hole row containing one feather of each treatment, feathers were randomly assigned a new position among feathers with the same dye, such that 10 series were created. For each trial, 4–6 observers ranked each series of two or three feathers within each dye group in descending order of the intensity of dye present. After ranking, the feathers were replaced in their original positions. Ranking procedures were conducted once per week for 8 wk.

We used repeated measures General Linear Model procedures (GLM; SAS Institute, Inc. 1988), with day or week as the repeated measure, to determine whether differences existed in dye retention using the various fixatives and fixative/carrier mixtures for each dye. If significant differences were detected, Tukey tests were used to determine which treatments differed.

Field Trials

All field trials were conducted during 1992 with Herring Gulls nesting on Turning Point Island (TPI), a series of breakwalls and rooftops within

or next to Sandusky Bay, Lake Erie, Erie County, Ohio (Dolbeer et al. 1990).

Application of dye mixture to eggs.—The dye mixture was made by first mixing 500 ml of heavy mineral oil with 60 g of the amorphous fumed silica. We then added 50 ml of 35% isopropyl alcohol and 37.5 g of RB.

We individually marked 260 Herring Gull nests with three-egg clutches on TPI using numbered 0.6-m wire surveying flags placed about 1 m from the nest. On 1 May, we placed 4–5 ml of the dye mixture on 50 clutches, distributing the dye evenly among the exposed surfaces of the three gull eggs using a small rubber spatula or wooden popsicle stick. We placed and spread the same amount of mixture on one egg within 100 clutches; the remaining 110 clutches served as controls. We monitored these nests once each week for 2 wk, then twice each week for an additional 4 wk to assess hatch success. During each visit we recorded nest number, treatment, number of eggs and number of chicks present. We defined hatch success as the number of chicks hatched divided by the total number of eggs within a treatment. Chi-squared tests of independence were used to assess whether there were any differences in hatch success among treatments.

We also compared the visibility of color-marks and hatch success using dye mixtures applied to dummy eggs placed in gull nests. During 7 and 13 May, on each of two breakwalls we individually marked 60 three-egg clutches using numbered wooden blocks placed flat within 1 m of the nests (Vermeer et al. 1988) or by painting a number on a rock adjacent to the nest. Each group of 60 nests was divided into three subgroups of 20 nests. We used brown domestic chicken eggs ($[\bar{x} \pm SD]$ $l = 58.67 \pm 1.77$ mm, $w = 43.53 \pm 0.96$ mm, $n = 20$) as dummy eggs. These eggs were approximately the mean length of the two egg sizes used by Cavanagh et al. (1992). The dye was applied to dummy eggs in a bead along the long axis using a grease gun, similar to Cavanagh et al. (1992). We placed a dummy egg treated with the RB and silica gel mixture into the nests of one pair of subgroups. The second pair of subgroups received a dummy egg with a similar amount of an RB and petroleum jelly mixture. This mixture was prepared as described by Cavanagh et al. (1992). We combined 428 g of RB and 1 l of water to form a paste which was mixed with petroleum jelly at a ratio of 315 g/kg. The third pair of subgroups received untreated dummy eggs and served as controls. All dummy eggs were placed in addition to existing gull eggs and were removed from nests 24 h after placement. Nest monitoring, data collection and analysis was identical to that done with field trials on TPI. On one breakwall, we also marked one gull egg in each of six three-egg clutches with 4–5 ml of the RB and silica gel mixture as done on TPI. We monitored these gulls in addition to those marked using dummy eggs at the breakwall at least once each week for 6 wk by recording the distance that birds marked were visible. We also recorded any behavior that appeared related to application of the dye mixtures.

Topical application.—We captured incubating Herring Gulls on TPI

and a rooftop using walk-in traps (Weaver and Kadlec 1970). Using a sponge brush, we topically applied a solution containing 3 g of MG per 60 ml of propylene glycol to the top of the heads of adults captured on TPI and to the breast and abdomen of those captured on the rooftop. We returned to these areas once or twice each week to assess retention and longevity of the dye.

RESULTS

Laboratory Trials

Effects of carriers on egg viability.—Hatch success of control eggs was significantly ($F = 4.18$; 8,15 df; $P < 0.01$) higher than hatch success of eggs treated with petroleum jelly or vegetable shortening at days 1 or 11. There were no significant differences ($P > 0.05$) between hatch success of any petroleum jelly or vegetable shortening treatments. No embryonic development was observed in any egg treated with either carrier at day 1. All embryos treated with a carrier at day 11 died ≤48 h after application. Silica gel affected embryonic growth similarly (31% hatch for control eggs; 0% hatch for eggs treated at days 1 and 11).

Vegetable shortening did not adhere to eggs as well as petroleum jelly or silica gel. Within 24 h of application, small pools of vegetable shortening had accumulated at the base of the eggs on the incubator trays. We concluded that vegetable shortening would not perform as well as petroleum jelly or silica gel and excluded it from further analyses.

Effects of fixatives on dye retention.—Use of fixatives significantly ($F = 44.69$; 5,54 df; $P < 0.01$) affected RB retention by feathers during trial 1. There was no difference ($P > 0.05$), however, among 70% isopropyl alcohol, 35% isopropyl alcohol and 2% acetic acid, and 2% acetic acid fixatives for RB retention (Table 1). These fixatives were superior to water alone or the 70% isopropyl alcohol and 2% acetic acid treatment. Increasing the amount of RB two-fold did not significantly ($P < 0.05$) improve dye intensity (treatments 1 and 2). For safety reasons and ease of mixing solutions, isopropyl alcohol was selected over acetic acid for use in trials 2–4.

Use of fixatives also significantly ($F = 13.79$–76.98; 5,54 df; $P < 0.01$) improved retention of all dyes during trials 2–4. There was no difference ($P > 0.05$) in retention of RB using 35% or 70% isopropyl alcohol or 99% propylene glycol during days 1 or 2, or days 1 and 2 combined (Table 2). Propylene glycol (99%) and isopropyl alcohol (35% and 70%) caused greatest retention of MG ($P < 0.05$). Isopropyl alcohol (35%) and propylene glycol (35%) caused greatest retention of PA ($P < 0.05$). Overall, retention of RB and MG increased with higher purity of propylene glycol and decreased with higher purity of isopropyl alcohol. Increasing purity of isopropyl alcohol and propylene glycol each caused increased retention of PA.

Effects of carriers on dye retention.—Silica gel improved overall retention

TABLE 1. Effects of acetic acid (AA) and isopropyl alcohol (IA) on retention of rhodamine-B (RB) dye on waterfowl feathers. Lower rank scores ($\bar{x} \pm SE$) indicate greater dye retention. Values within a column with different letters are significantly different (Tukey tests, $P < 0.05$). Water was used as the diluent for all 60-ml solutions.

Solution	Day 1	Day 2	Combined
6 g RB	5.2 ± 0.10 C	5.2 ± 0.11 D	5.2 ± 0.07 B
3 g RB	4.2 ± 0.14 B	4.5 ± 0.14 C	4.4 ± 0.10 B
3 g RB, 70% IA	2.1 ± 0.12 A	2.1 ± 0.10 AB	2.1 ± 0.08 A
3 g RB, 70% IA, 2% AA	5.3 ± 0.10 C	4.9 ± 0.14 CD	5.1 ± 0.08 B
3 g RB, 35% IA, 2% AA	2.3 ± 0.13 A	2.4 ± 0.13 B	2.4 ± 0.09 A
3 g RB, 2% AA	1.9 ± 0.13 A	1.8 ± 0.14 A	1.9 ± 0.10 A

of all three dyes by feathers ($F = 54.78–746.19$; 1,18 or 2,27 df; $P < 0.01$; Fig. 1). There was no significant ($F = 1.58$; 8,144 df; $P = 0.14$) day × treatment interaction for PA. There were significant day × treatment interactions with MG ($F = 4.46$; 8,144 df; $P < 0.01$) and RB ($F = 8.34$; 16,216 df; $P < 0.01$) dyes, however. The interaction detected for MG was a consequence of no variance within treatments during weeks 0–2 and 4. Of the two RB and petroleum jelly mixtures, the mixture containing 35% isopropyl alcohol had overall greater retention ($P < 0.05$). The RB treatment containing silica gel had greatest visibility through week 5. By week 8, intensity of dye from all RB treatments appeared similar. Feathers containing MG and PA treatments with silica gel consistently retained a greater proportion of dye than treatments with petroleum jelly.

Field Trials

Application of dye mixtures to eggs.—Application of the RB and silica gel mixture significantly ($\chi^2 = 249.88$, 2 df, $P < 0.01$) reduced hatch success (Table 3). Hatch success for untreated clutches (70.8%) was >4 times greater than was hatch success of clutches with a single gull egg treated (16.8%). Hatch success for clutches with one and three marked gull eggs also differed ($\chi^2 = 12.18$, 1 df, $P < 0.01$; 16.8% and 4.1%,

TABLE 2. Pooled effects of 2-d test of propylene glycol (PG) and isopropyl alcohol (IA) on retention of Rhodamine B, Malachite Green and Picric Acid dyes on waterfowl feathers. Lower rank scores ($\bar{x} \pm SE$) indicate greater dye retention. Values within a column with different letters are significantly different (Tukey tests, $P < 0.05$).

Fixative[a]	Rhodamine B	Malachite Green	Picric Acid
IA (35%)	2.3 ± 0.11 A	2.9 ± 0.13 AB	1.6 ± 0.05 A
IA (70%)	2.5 ± 0.16 A	2.5 ± 0.10 AB	2.7 ± 0.09 B
IA (99%)	4.5 ± 0.12 B	4.5 ± 0.13 CD	4.7 ± 0.06 C
PG (35%)	4.9 ± 0.09 B	5.7 ± 0.05 D	1.9 ± 0.08 AB
PG (70%)	4.5 ± 0.15 B	3.4 ± 0.11 BC	4.2 ± 0.07 C
PG (99%)	2.4 ± 0.12 A	2.0 ± 0.14 A	5.9 ± 0.03 D

[a] Each 60-ml solution contained either IA or PG and water as the diluent.

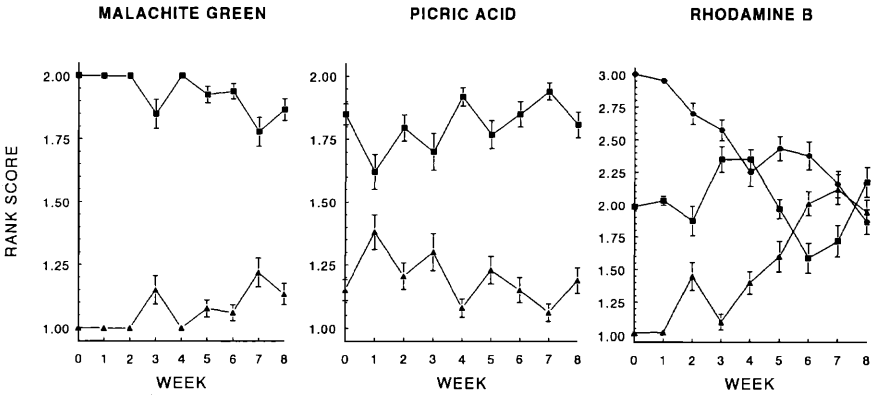

FIGURE 1. Effects of petroleum jelly and oil-based silica gel carriers on retention of Malachite Green, Picric Acid and Rhodamine B dyes by waterfowl feathers. Square symbols represent treatments containing petroleum jelly, triangles represent treatments containing silica gel and circles represent the RB and petroleum jelly mixture used during 1991. See text for detailed description of dye mixtures. Lower rank scores indicate greater dye retention ($\bar{x} \pm SE$). Silica gel caused overall greater retention for all dyes ($P < 0.05$).

respectively). Only 1 of 85 clutches produced three chicks from nests with one marked egg as compared to 46 of 105 clutches from control nests.

Dye mixtures applied to dummy eggs and placed in gull nests for 24 h also significantly ($\chi^2 = 25.27$, 2 df, $P < 0.01$) reduced hatch success (from 67.5 to 37–41%, Table 3). Hatch success was similar between mixtures containing silica gel and petroleum jelly ($\chi^2 = 1.11$, 1 df, $P = 0.29$). The number of clutches that produced one, two or three chicks was also similar. Hatch success of control clutches with dummy eggs was similar to control clutches without dummy eggs (67.5% vs. 70.8%, respectively).

All eggs in each clutch received at least a small amount of the dye mixture within 24 h after marking, irrespective of treatment, as a result of gulls transferring dye from one egg to another via their plumage. The entire surface of most eggs had a dark pink hue. Unmarked eggs from clutches where one egg was marked were less intensely colored than eggs to which dye was directly applied.

Gull behavior.—Gulls returned to their nests within 30 min of marking. Upon arrival, gulls walked near the nest or stood on the rim looking at the eggs for several minutes, often removing a portion of the dye with their bills before initiating incubation.

Gulls ingested both dye mixtures frequently, as evidenced by dyeing of their bills and the presence of numerous droppings and regurgitations with pink stains shortly after marking. Gulls were observed removing dye mixtures applied to dummy eggs more frequently than dye applied to gull eggs. Several gulls were observed picking up portions of the dye with their bills and throwing it from the nest site. The bead of dye on

TABLE 3. Hatching success of three-egg clutches of Herring Gulls after application of 4–5 ml of Rhodamine B and silica gel or petroleum jelly dye mixture, Sandusky Bay, Lake Erie, Erie Co., Ohio, 1992.

Treatment[a]	# clutches	% nests with 0 chicks	1 chick	2 chicks	3 chicks	% hatch success[b]	Chicks/ nest
Silica gel mixture applied to three gull eggs/clutch	41	88	10	2	0	4.1	0.1
Silica gel mixture applied to one gull egg/clutch	85[c]	60	28	11	1	16.8	0.5
Control clutches	105	8	16	32	44	70.8	2.1
Silica gel mixture applied to a dummy egg (24 h)	40	35	25	23	18	40.8	1.2
Petroleum jelly mixture applied to a dummy egg (24 h)	40	40	23	25	13	36.7	1.1
Control clutches (dummy egg in nest for 24 h)	40	0	23	53	25	67.5	2.0

[a] See text for detailed description of treatments.
[b] Number of chicks hatched/number of eggs.
[c] Includes one four-egg clutch.

the dummy eggs appeared easier to remove than dye spread over a larger portion of the gull eggs.

We found two dead adult gulls on TPI at nests where dye was applied to one egg of the clutch. One had small amounts of the dye mixture on its bill, the other on its breast. Although we observed dead unmarked gulls during the same time period, none were located at a nest.

Retention and visibility of dye mixtures.—Within 3 h after dye application, both members of a pair were marked. The first bird of a pair to incubate was marked extensively, with dye covering most of the breast and abdomen area. In addition to marked bills and feet, the wrists were also frequently marked from folding them against the body. The second member of each pair was marked considerably less, with dye generally covering only the brood patch area. For all treatments, the area dyed increased initially in size from preening and contact with water which activated the dye.

Within 24 h of marking, at least some birds of each treatment were visible at about 400 m using 8× binoculars. The color-mark was more pronounced on gulls to which dye was applied to one gull egg, appearing more intense and covering a larger area of the bird than those dyed using dummy eggs. Of gulls marked using dummy eggs, virtually no assumed second-marked pair members were visibly marked after 3 wk. Some first-marked birds were visible to 350 m using a 15× spotting scope after 3 wk, although no birds dyed using petroleum jelly were visible at this distance using 8× binoculars. No gulls marked via dummy eggs were

visibly dyed after 5 wk when viewed from 100 m using 8× magnification. Few gulls marked via dye applied to gull eggs were visibly dyed after 6 wk, although an occasional marked gull was observed 10 wk post-marking.

Topical application.—We marked 32 Herring Gulls using the MG and propylene glycol mixture (20 on top of the head, 12 on the breast and abdomen). No gulls were observed to retain the dye greater than 2 wk. For both locations of application, about 30% of the gulls retained their mark after 1 wk and <10% retained their mark for 2 wk. The dye applied to the breast and abdomen was more visible than dye applied to the top of the head. We did not observe enlargement of the dyed area due to preening or contact with water as observed on gulls marked using RB.

DISCUSSION

Isopropyl alcohol and propylene glycol were each more effective in increasing retention of dyes than water alone. These fixatives likely disperse oil that occurs naturally on feathers, allowing the dye to attach directly to them. The oil's repellency of water results in the inability of the dye in a water solution to attach to the feathers, causing minimal or no dyeing and facilitating removal by a bird.

We concur with Cavanagh et al. (1992) that marking gulls via transfer of dye from eggs appears better suited for water soluble dyes such as RB. In contrast to gulls marked with MG, those marked with RB were able to spread the dye over an area larger than the area of contact, which improved visibility. This likely reduces the longevity of the color-mark, however, particularly with gulls or other birds that are often found on water, by allowing frequent contact with a solvent (water).

Silica gel adhered to feathers better than petroleum jelly. Although not quantified, silica gel also appeared to adhere to eggs better than petroleum jelly. These carriers have been used similarly elsewhere for marking gulls and cattle egrets (Cavanagh et al. 1992, Paton and Pank 1986). Paton and Pank (1986) observed no embryonic mortality using an RB and oil-based silica gel mixture, but recommended further research. Cavanagh et al. (1992) applied an RB and petroleum jelly mixture to dummy eggs that were placed in gull nests for ≥24 h. They reported low levels of adult mortality and nest destruction but presented no data on egg mortality. Carriers caused 100% embryonic mortality during our laboratory trials and during field studies caused significant ($P < 0.01$) reductions in hatch success, however. Other petroleum distillates have also been reported to reduce hatch success of gull eggs (Blokpoel and Hamilton 1989, Christens and Blokpoel 1991, Morris and Siderius 1990).

The lower mortality of gull eggs in nests with treated dummy eggs compared to treated gull eggs may be attributed to dummy egg treatments occurring 1–2 wk after the gull egg treatments. Studies evaluating effects of other petroleum distillates on embryonic mortality have found improved hatch success when eggs were treated later in incubation (Albers 1978, Lewis and Malecki 1984). Thus, the mortality of gull eggs in nests treated with dyed dummy eggs may have been greater than we measured (and

comparable to the one-egg gull treatment) if the treatment had occurred earlier in incubation.

Our results have important implications for biologists intending to use similar materials and methods. Marking gulls via their eggs will likely be unsuitable for some studies involving reproduction, behavior, foraging and movements. Gull behavior will be modified during chick-rearing if hatching success is reduced or does not occur. Gull movements will also be influenced, particularly foraging movements. Gulls are reported to have different foraging and movement patterns between incubation and chick-rearing (Annett and Pierotti 1989, Pierotti and Annett 1987).

In conclusion, color marking via dye transferred from eggs is a cost effective and efficient technique for short-term marking of large numbers of incubating adult Herring Gulls. Significant reductions in hatch success and subsequent modified behavior decrease its utility, however. If biologists intend to use this technique, we recommend using a mixture of 37.5 g RB and 50 ml isopropyl alcohol (35%) with about 500 ml of an oil-based silica gel to increase the amount of dye transferred to the adults and improve the longevity of the color-mark. If an additional color-mark is required, use 37.5 g MG and 50 ml propylene glycol with the carrier. We also recommend applying the dye mixture to a dummy egg or to only one egg of a clutch during late incubation to reduce embryonic mortality. Even under these conditions, however, some mortality is likely to occur.

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