J. Field Ornithol., 71(4):694–701

RHODAMINE-INJECTED EGGS TO PHOTOGRAPHICALLY IDENTIFY SMALL NEST-PREDATORS

THOMAS J. MAIER AND RICHARD M. DEGRAAF

USDA Forest Service, Northeastern Research Station, 201 Holdsworth Hall
University of Massachusetts, Amherst, Massachusetts 01003 USA

Abstract.—Photographs that clearly disclose avian-nest predators are difficult to obtain, particularly when predators are small and exhibit subtle depredatory behavior. We exposed House Sparrow (*Passer domesticus*) eggs injected with Rhodamine B dye in camera-monitored ground nests for 12-d periods at 76 sites within mixed-hardwood forest stands in central Massachusetts, June–July 1997. Dye-injected eggs enabled us to recognize with certainty when eggs were breached at the nest because their contents were fluorescent pink and readily detected photographically. Eleven potential predator species were identified disturbing nests, of which eight were confirmed as predators. Eastern chipmunks (*Tamias striatus*) were the most frequent predators detected, along with fisher (*Martes pennanti*), raccoon (*Procyon lotor*), Blue Jays (*Cyanocitta cristata*), Black-capped Chickadees (*Poecile atricapillus*), red squirrels (*Tamiasciurus hudsonicus*), an Eastern Towhee (*Pipilo erythrophthalmus*), and a white-footed mouse (*Peromyscus leucopus*). White-footed mice were the most commonly detected species disturbing nests, but were photographed only once actually destroying an egg. The visual cue provided by dye-injected House Sparrow eggs confirmed depredatory behavior by eastern chipmunks, Black-capped Chickadees, an Eastern Towhee, and a white-footed mouse.

HUEVOS INYECTADOS CON EL TINTE RODAMINA B PARA IDENTIFICAR FOTOGRAFICAMENTE DEPREDADORES PEQUEÑOS

Sinopsis.—Es difícil obtener fotografías que claramente identifiquen depredadores de nidos de aves. Utilizanos huevos de gorrión (*Passer domesticus*), injectados con el tinte rodamina B, para identificar a los depredadores en nidos monitoreados con cámaras fotográficas. De junio a julio del 1977 se monitorearon, por un periodo de 12 d, 76 localidades con nidos en un bosque mixto de maderas duras en Massachussetts. El tinte injectado en los huevos permitió identificar con claridad cuando los huevos eran rotos, ya que dicho tinte es fluorescente y aparece en las fotografías. Once depredadores potenciales fueron identificados causando disturbios en nidos, y ocho especies (cinco de mamíferos y tres de aves) como depredadores de los huevos. La ardillita (*Tamias striatus*) fue identificada como el depredador principal, mientras que el ratoncito (*Peromyscus leucopus*) fue la especie identificada como el causante del mayor número de disturbios. Este fue fotografiada solamente una vez depredando huevos. Los huevos injectados con tinte permitieron confirmar depredación por parte de mamíferos tales como *Tamias striatus, Peromyscus leucopus* y pájaros como *Poecile atricapillus* y *Pipilo erythrophthalmus.*

The inability to identify species responsible for significant portions of passerine nest predation may be largely due to difficulties involved in identifying small nest-predators and their effects (Roper 1992, Haskell 1995, DeGraaf and Maier 1996). Although such predators are often relatively abundant and, as such, frequently detected at camera-monitored nests, their depredatory behavior may be as subtle as egg-holing by rodents (Maxson and Oring 1978) or egg-pecking by small birds (Pickman and Belles-Isles 1988). In such cases, photographs may infrequently disclose the initial predation event and prevent objective documentation of depredation, as other predators often visit the same nests (Reitsma et al. 1990) and obliterate evidence of original predation (Larivière 1999). Giv-

en that placing cameras closer to nests may either scare predators away or force the abandonment of natural nests (Thompson et al. 1999), we tested Rhodamine B dye-injected House Sparrow (*Passer domesticus*) eggs in camera-monitored artificial ground nests to determine if such eggs would provide a supplementary visual cue when breached by potential small predators. In tests, when a dye-injected egg was breached, the fluorescent pink contents were more visible than naturally clear albumen, thus increasing our chances of photographically confirming its depredation at the nest.

METHODS

We placed 76 camera-monitored artificial ground-nests in mature forest stands for 12-d periods during June–July 1997. All stands were at least 80 yr old and of the red oak (*Quercus rubra*)-white pine (*Pinus strobus*)-red maple (*Acer rubrum*) forest-cover type (Eyre 1980). The stands were scattered throughout a 630-km^2 extensive, managed mixed-wood forest encompassing Quabbin and Ware River watersheds in central Massachusetts. Nests were separated from each other by at least 100 m, with an average separation of 3 km.

Nests were aviculturists' wicker baskets, 10 cm in diameter and 6-cm deep, weathered for 3 wk prior to their deployment. Each nest contained a single House Sparrow egg that had been injected with Rhodamine B; a reddish, non-toxic dye with various wildlife applications (Nietfeld et al. 1994). House Sparrow eggs are approximately the mean egg width for eastern Neotropical migrants (Haskell 1995: Fig. 1) and have color and markings similar to many small passerines (Harrison 1975). Maier and DeGraaf (2000) provide descriptions of the cameras, color film, camera-trigger mechanism, placement, and installation procedures used in this study. Cameras featured a time-date function.

House Sparrow eggs were gathered locally and refrigerated immediately prior to use within the week. All eggs were rinsed in well water, air-dried, and injected with 0.03 cc of a Rhodamine B and ethyl alcohol mixture (5%) immediately prior to their placement. Injection was made through the egg's air cell (blunt end) and into the albumen by slowly twirling a 1 cc, 0.45 mm × 10 mm insulin syringe, and the injection site was sealed with white correction fluid. Surgical gloves were worn while preparing the eggs. The Rhodamine B (generic name) dye was powdered Neptun Red Base NB 543, supplied by BASF Corporation, Rensselaer, New York. (Trade names and commercial products are mentioned for information only; no endorsement by the U.S. Department of Agriculture is implied.)

Injecting eggs with the Rhodamine B-alcohol mixture could influence nest predation. To address this treatment as a potential bias, we conducted an accessory experiment in 1998 to see if treated eggs were depredated at a different frequency than untreated eggs. We exposed 40 pairs of dye-injected and natural House Sparrow eggs (the latter as controls) in separate ground nests within forest stands randomly selected from

those used in 1997. All nest pairs were separated by at least 100 m, with each pair's nests separated by 50 m. Our methods were the same as in 1997, except cameras were not used to monitor nest activity. We used a two-tailed binomial test to compare the frequency of depredation between treatments, using $P = 0.5$ as our null hypothesis, with a significance level of $P < 0.05$ (Zar 1996:530). Egg pairs ($n = 40$) were depredated in the following manner: both treated and control eggs, 26 cases; only treated eggs, 8 cases; only control eggs, 3 cases; neither egg depredated, 3 cases. The predation frequency of dye-injected eggs was not significantly different from that of natural eggs (two-tailed binomial: $P = 0.23$) in the 1998 accessory experiment. We also compared predation for treated and untreated eggs from 1997 and 1998 by applying a *G*-test to a 2×3 contingency table (Zar 1996:502).

All nests were checked after a 12-d period, approximately the mean incubation time for small forest passerines (Harrison 1975). Eggs found out of the nest, destroyed in the nest, or missing were considered predation events. For the purposes of this study, we define "predation" as any activity that would destroy a viable egg (i.e., we include egg-pecking behavior that has resulted in a breached egg). Eggs found off their trigger, but still in the nest and sound, were considered "disturbed."

Color photographs, along with any corroborative remains of depredated eggs, were used to identify predator species. Rhodamine-injected eggs facilitated predator identification when eggs were breached at the nest site because the fluorescent pink egg contents were more readily visible in the color photographs than natural egg white. Egg remains alone may not indicate predator identity (Larivière 1999), thus remains were only used as supportive evidence to photographs and then only when they retained diagnostic value for identifying depredation by either small mammals or small passerines (see Maier and DeGraaf 2000). Our decisions on whether to classify an animal as an egg predator were conservative. For example, a photograph of an eastern chipmunk (*Tamias striatus*) with an egg in its mouth or holding a breached egg with dye visible was taken as evidence of predation, whereas a chipmunk holding an egg without visible dye (but later found depredated) was considered a predation event by that animal only if egg remains were supportive (e.g., the egg shell had a 4–8-mm diameter hole in the blunt end). This predator identification protocol was necessary because we photographed chipmunks holding eggs in their forelimbs without subsequent egg predation. For this reason, we differentiate between potential predators disturbing nests and "confirmed" predators destroying eggs.

RESULTS

Eleven potential predator species (eight mammal and three bird) were identified disturbing 87% of all nests disturbed ($n = 63$) (Table 1). White-footed mice (*Peromyscus leucopus*) were the most frequently detected, representing 35% of recorded animals identified to species ($n = 55$), with eastern chipmunks the next most frequent (25%).

TABLE 1. Species identified disturbing and confirmed depredating 76 camera-monitored artificial ground-nests containing Rhodamine B dye-injected House Sparrow (*Passer domesticus*) eggs in central Massachusetts mixed-wood forests, June and July 1997.

Species	Number of nests disturbed	Number of nests with confirmed predation
White-footed mouse (*Peromyscus leucopus*)	19	1
Eastern chipmunk (*Tamias striatus*)	14	7
Blue Jay (*Cyanocitta cristata*)	4	4
Fisher (*Martes pennanti*)	4	4
Raccoon (*Procyon lotor*)	4	4
Black-capped Chickadee (*Poecile atricapillus*)	3	3
Red squirrel (*Tamiasciurus hudsonicus*)	3	2
Eastern Towhee (*Pipilo erythrophthalmus*)	1	1
Eastern Gray squirrel (*Sciurus carolinensis*)	1	0
Porcupine (*Erethizon dorsatum*)	1	0
Masked shrew (*Sorex cinereus*)	1	0
Suspected small mammal[a]	6	6
Suspected large mammal[b]	1	1
Unidentified	1	26
No disturbance	13	NA
Total	76	59

[a] Photographs revealed eggs held by chipmunks with forelegs and egg fragments were strongly characteristic of small mammal depredation, e.g., 0.4–0.8 cm hole in blunt end.
[b] Nest set was destroyed.

Eight species, five mammal and three bird, were confirmed as predators, and were responsible for 44% of all cases of predation (n = 59) (Table 1). Eastern chipmunks were the predominant predator, representing 27% of those identified to species (n = 26), followed by Blue Jays (*Cyanocitta cristata*), fisher (*Martes pennanti*), and raccoon (*Procyon lotor*), each 15%. Most nests were depredated (78%), with most identifiable predation (55%, n = 33) attributed to small predators, i.e., mice, chipmunks, Black-capped Chickadees (*Poecile atricapillus*), an Eastern Towhee (*Pipilo erythrophthalmus*), and unidentified small mammals. Approximately 70% of the unidentified predation events (n = 26) were attributed to equipment failure (i.e., moisture in cameras or short-circuited camera-triggers) caused by persistent heavy rains during nest exposure periods.

Our use of dye-injected House Sparrow eggs allowed us to confirm additional egg depredation behavior by four chipmunks, two Black-capped Chickadees, one female Eastern Towhee, and one white-footed mouse. Eastern chipmunks were photographed in three cases sitting next to nests on their hindquarters holding breached eggs with their forelimbs and in one case pinning a breached egg against the side of a nest. In two cases, Black-capped Chickadees perched on nest rims, pecking at eggs repeatedly; in both cases, the hole in the egg was enlarged, becoming more visible in successive photographs due to the dyed contents. A female Eastern Towhee was photographed perched on a nest rim in two succes-

sive photographs; in the first it was apparently pecking at the egg and in the second had dyed egg remains on her beak. The single case of predation by a white-footed mouse was depicted by 12 photographs spanning 185 min. The first six photographs, spanning 5 min, were of a large adult mouse in a nest mouthing and moving the undamaged egg around; the second six photographs, spanning 5 min, were of an apparently identical mouse (possibly the same individual) that had breached the egg. Almost 3 h separated the two series of photographs.

We photographed more than one species of potential predator at 43% of the nests where identifiable activity was recorded ($n = 55$), detecting a maximum of four potential predator species at one nest site (in the order of their detection: raccoon [responsible for predation], white-footed mouse, chipmunk, and fisher). All three chickadee and the towhee depredations were followed by other potential predator species (i.e., white-footed mice, chipmunks, and raccoon). Of these four nests, one contained shell fragments of no diagnostic value, one was destroyed along with its camera cable, and two had no apparent egg remains in the vicinity.

The predation rate of dye-injected House Sparrow eggs in 1997 (78%) was not significantly different from that of either natural eggs (73%) or treated eggs (85%) in our 1998 experiment ($G = 1.92$, $P = 0.38$).

DISCUSSION

Subtle damage to natural eggs caused by small predators may infrequently be apparent in photographs, but we could often determine that eggs were breached because the dyed fluorescent pink egg contents were readily distinguishable on color prints. Moreover, in some cases, the damage to the egg was made sufficiently explicit by the dye to reveal how depredation was accomplished, thus providing insight on predator behavior and how natural egg depredation may occur. Injecting House Sparrow eggs with Rhodamine B dye enabled us to photographically document egg depredation in artificial nests by small species either not previously considered responsible (e.g., Eastern Towhee) or considered potential nest predators, though seldom confirmed as such (e.g., *Peromyscus* spp.; Maxson and Oring 1978, Murray et al. 1983, Keyser et al. 1998). Additionally, our use of dye-injected eggs more than doubled the frequency of confirmed predation by eastern chipmunks and Black-capped Chickadees.

Eight species were confirmed as predators of artificial nests in this study. Blue Jay, fisher, raccoon, and red squirrel (*Tamiasciurus hudsonicus*) have often been documented as predators of bird eggs. Eastern chipmunks have been considered potential avian nest predators from narrative accounts (Pettingill 1976, Elliot 1978, Snyder 1982 and references therein), and their relative abundance has been tenuously associated with the nest failure of Dark-eyed Juncos (*Junco hyemalis*) (Ketterson et al. 1996). More recent observations, however, have led some researchers to surmise that chipmunks are not important nests predators. Sloan et al.

(1998) photographed chipmunks at only 5% of artificial nests in similar hardwood habitat in New Hampshire, and extensive, multi-year observations of chipmunk behavior in Pennsylvania failed to reveal a single nest predation event (C. Mahan, pers. comm.). Nevertheless, in this study, chipmunks were the most frequently detected nest predators. Black-capped Chickadees have been observed displaying depredatory egg-pecking behavior (Picman and Belles-Isles 1988), but to the best of our knowledge have been documented destroying a real egg in an artificial nest only once previously (Maier and DeGraaf 2000). We were unable to discern whether chickadees were consuming eggs or exhibiting interspecific egg destruction, but they appeared to be persistent in their activity and in all three cases of detection, depredation was confirmed. We found no previous record of Eastern Towhee exhibiting interspecific egg-pecking behavior, but have photographed this species, female in all cases, at artificial nests previous to this study (Maier and DeGraaf 2000). In those previous cases, House Sparrow eggs were depredated, but we were unable to confirm predator identity. White-footed mice have been implicated as nest predators (Kent 1984, Guillory 1987), but not previously documented actually destroying a real egg in an artificial nest. Paradoxically, although white-footed mice were our most frequently detected species, we confirmed only a single case of predation. This fact, plus the apparently laborious predation efforts depicted in our photographs, suggests that white-footed mice are infrequent predators of Neotropical passerine eggs the size of House Sparrow eggs.

Indisputable identification of small avian-nest predators or their effects is difficult. The mere presence of an animal at a nest, whether photographed or evidenced by resultant sign, does not confirm its activity as a predator (Major and Gowing 1994) irrespective of its frequency of detection (see Table 1). Documentation of the actual destruction of a nest or its contents, however, does imply depredatory behavior and may be critical because physical evidence at depredated nests may be destroyed by subsequent visits of many other potential predators. In this study, many of the nests were visited by multiple species capable of depredating (by removal or breach) House Sparrow eggs, after photographic documentation of egg depredation by small predators.

Our intent was to identify actual predation by small nest predators rather than to document rates of nest predation; however, almost all nests were disturbed and most were depredated. Did the mixture of Rhodamine B and ethyl alcohol in the eggs attract animals or facilitate depredation? Treated eggs may have been more apparent to predators, as the dye lent a light pink cast to the shell. Further, Rhodamine B is fluorescent and many avian species, along with some rodent species, appear to be capable of seeing wavelengths in the near UV (Bennet and Cuthill 1994 and references therein). Ethyl alcohol may have been an olfactory attractant after permeating the eggshell (Romanoff and Romanoff 1949, Burley and Vadehra 1989), because many species exhibit an affinity for alcohol (Siegel 1989). Finally, piercing the eggshell with the injection may have

weakened its structural integrity. Nevertheless, the treatment bias test performed in 1998 failed to reveal a significant difference between the depredation of treated and natural eggs, and predation rates for 1997 and 1998 were similar. Although our use of dye-injected House Sparrow eggs enabled us to document depredation by small predators, further work with similar detection techniques that supplement photography is necessary to elucidate the roles of small avian-nest predators and their behavior at passerine nests.

ACKNOWLEDGMENTS

We thank M. Stoddard and N. Perry for assistance with fieldwork; S. Drawbridge, H. Eck, D. Small and B. Spencer for assistance in forest site selection; K. Doyle, T. Fuller, W. Healy, P. Sievert, and two anonymous reviewers for their comments on the manuscript; S. Cooter for ethological instruction; and M. Sheremeta for typing and proofing the final manuscript. We also thank the Metropolitan District Commission, Division of Watershed Management, Quabbin section, for access to their management area.

LITERATURE CITED

BENNET, A. T. D., AND I. C. CUTHILL. 1994. Ultraviolet vision in birds: what is its function? Vision Research 34:1471–1478.

BURLEY, R. W., AND D. V. VADEHRA. 1989. The avian egg: chemistry and biology. John Wiley and Sons, New York. 472 pp.

DEGRAAF, R. M., AND T. J. MAIER. 1996. Effect of egg size on predation by white-footed mice. Wilson Bull. 108:535–539.

ELLIOT, L. 1978. Social behavior and foraging ecology of the eastern chipmunk (*Tamias striatus*) in the Adirondack Mountains. Smithson. Contrib. Zool. 265:1–107.

EYRE, F. H. 1980. Forest cover types of the United States and Canada. Soc. of American Foresters, Washington, D.C. 148 pp.

GUILLORY, H. D. 1987. Cavity competition and suspected predation on Prothonotary Warblers by *Peromyscus* spp. J. Field Ornithol. 58:425–427.

HARRISON, H. H. 1975. A field guide to birds' nests. Houghton Mifflin, Boston, Massachusetts. 257 pp.

HASKELL, D. G. 1995. Forest fragmentation and nest predation: are experiments with Japanese Quail eggs misleading? Auk 112:767–770.

KENT, G. N. 1984. Eastern Bluebird victim of agonistic behavior by white-footed mouse. Sialia 6:3–7.

KETTERSON, E. D., V. NOLAN, JR., M. J. CAWTHORN, P. G. PARKER, AND C. ZIEGENFUS. 1996. Phenotypic engineering: using hormones to explore the mechanistic and functional bases of phenotypic variation in nature. Ibis 138:70–86.

KEYSER, A. J., G. E. HILL, AND E. C. SOEHREN. 1998. Effects of forest fragment size, nest density, and proximity to edge on the risk of predation to ground-nesting passerine birds. Conserv. Biol. 12:986–994.

LARIVIÈRE, S. 1999. Reasons why predators cannot be inferred from nest remains. Condor 101:718–721.

MAIER, T. J., AND R. M. DEGRAAF. 2000. Predation on Japanese Quail vs. House Sparrow eggs in artificial nests: small eggs reveal small predators. Condor 102:325–332.

MAJOR, R. E., AND G. GOWING. 1994. An inexpensive photographic technique for identifying nest predators at active nests of birds. Wildl. Res. 21:657–666.

MAXSON, S. J., AND L. W. ORING. 1978. Mice as a source of egg loss among ground-nesting birds. Auk 95:582–584.

MURRAY, K. G., K. WINNETT-MURRAY, Z. A. EPPLEY, G. L. HUNT, JR., AND D. B. SCHWARTZ. 1983. Breeding biology of the Xantus' Murrelet. Condor 85:12–21.

NIETFELD, M. T., M. W. BARRET, AND N. SILVY. 1994. Wildlife marking techniques. Pp. 140–

168, *in* T. A. Bookhout, ed. Research and management techniques for wildlife and habitats. 5th ed. Wildlife Society, Bethesda, Maryland. 740 pp.

PETTINGILL, O. S., JR. 1976. Observed acts of predation on birds in northern Lower Michigan. Pp. 33–41, *in* D. A. Lancaster and J. R. Johnson, eds. The Living Bird: Fifteenth Annual of the Cornell Laboratory of Ornithology. Laboratory of Ornithol., Cornell University, Ithaca, New York.

PICMAN, J., AND J. BELLES-ISLES. 1988. Interspecific egg pecking by the Black-capped Chickadee. Wilson Bull. 100:664–665.

REITSMA, L. R., R. T. HOLMES, AND T. W. SHERRY. 1990. Effects of removal of red squirrels, Tamiasciurus hudsonicus, and eastern chipmunk, Tamias striatus, on nest predation in a northern hardwood forest: an artificial nest experiment. Oikos 57:375–380.

ROMANOFF, A. L., AND A. J. ROMANOFF. 1949. The avian egg. John Wiley & Sons, New York. 918 pp.

ROPER, J. J. 1992. Nest predation experiments with quail eggs: too much to swallow? Oikos 65:528–530.

SIEGEL, R. K. 1989. Intoxication: life in pursuit of artificial paradise. E. P. Dutton, New York. 390 pp.

SLOAN, S. S., R. T. HOLMES, AND T. W. SHERRY. 1998. Depredation rates and predations of artificial bird nests in an unfragmented northern hardwoods forest. J. Wildl. Manage. 62:529–539.

SNYDER, D. P. 1982. Tamias striatus. Mammalian Species 168:1–8.

THOMPSON, F. R. III. W. DIJAK, AND D. E. BURHANS. 1999. Video identification of predators at songbird nests in old fields. Auk 116:259–264.

ZAR, J. H. 1996. Biostatistical analysis. 3rd ed. Prentice-Hall, Englewood Cliffs, New Jersey.

Received 15 Jun. 1999, accepted 19 Nov. 1999.