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How do Predators Locate Nests of Ornate Box Turtles (*Terrapene ornata*)? A Field Experiment

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Abstract.—**Turtles are subject to high rates of nest destruction from mesopredators. Scents associated with nest contents, proximity to woody edge, and marker flags are suggested cues influencing nest predation. With few exceptions, most turtle nest predation studies involve aquatic turtles. During studies of terrestrial Ornate Box Turtles (*Terrapene ornata*), we documented high rates of nest predation. We designed an experiment to simultaneously compare combinations of factors which may influence predation using artificial nests on two sand prairies used for nesting. Artificial nests contained treatments arranged in 1 1 m2 plots: disturbed soil with a buried Chicken (*Gallus gallus*) egg marked with a flag; disturbed soil without an egg, but marked with a flag; disturbed soil with a buried egg and no flag; and undisturbed soil marked with a flag. We established replicate transects of arrays perpendicular to the woody edge of the prairie and observed excavation for 24 d each in June, July, and August. Disturbed soil was the only significant predictor of excavation. With the exception of the undisturbed soil with flag, effectively untouched by predators for 22–24 d, average nest survival was low (range: 2–3 d). There were no significant differences in destruction of artificial nests between distances from the woody edge and artificial nests. Because undisturbed soil did not elicit digging, we suggest that nesting in soft sandy soil is not only adaptive because of ease of excavation, but rain and wind also hide surface evidence and/or odor of digging quickly and, therefore, obscure the nest.**

Key Words.—nest predation; nest survival; turtle nests

Introduction

Nest predation is a significant source of mortality in turtles (Schwanz et al. 2010), and the loss of eggs, embryos, or young offspring to predators lowers recruitment (Strickland et al. 2010) and population growth. North American predators of turtle nests include Raccoons (*Procyon lotor*), Striped Skunks (*Mephitis mephitis*), and Red Foxes (*Vulpes vulpes*; Hamilton 1940; Wilhoft et al. 1979), with Raccoons being perhaps the most important (e.g., Wilhoft et al. 1979; Christiansen and Gallaway 1984; Mitchell and Klemens 2000; Marchand and Litvaitis 2004; Strickland et al. 2010). Populations of mesopredators, omnivorous medium-sized predators such as Raccoons, are enhanced by anthropogenic modifications to the environment including, but not limited to, food supplementation and creation of habitat edges. Mesopredators, such as Raccoons are more abundant in modern landscapes than in the past, which could negatively affect population viability for some turtles (McDougal 2000; Hamilton et al. 2002; Troyer et al. 2014).

Predators may use both visual and/or olfactory cues to locate turtle nests (Zeveloff 2002; Conover 2007), but the relative importance of these and other cues may vary among species and locations. The study of cues used by predators to detect nests is complicated by predator sensitivity to the presence and activities of researchers in the field. For example, field researchers often use a marking system to help them locate particular nests, and some nest predators may use the marking system to locate nests. Corvids learned to recognize Popsicle^{TM} stick markers used by researchers to identify nest locations (Rollinson and Brooks 2007). However, mammalian nest predators were not sensitive to the surveyors' flags used to mark nests in other studies (Tuberville and Burke 1994; Burke et al. 2005). Some researchers suggest that predators use olfactory cues to find turtle nests. For example, Legler (1954) thought that the urine released by female Painted Turtles (*Chrysemys picta*) during nesting actually provided olfactory cues that predators used to locate nests, and additional studies suspected Raccoons and Red Foxes used olfaction to located turtle nests (Congdon et al. 1983; Spencer 2002). Some researchers (Legler 1954; Burke et al. 1998; Bowen and Janzen 2005) proposed that rainfall the night of nesting may dissipate or eliminate nest odor stimuli. However, other researchers found that soil disturbance alone from nesting was enough for predators to locate turtle nests (Burke et al. 2005; Strickland et al. 2010).

In addition to odors left by nesting turtles, mammalian predators may use human scent left by researchers to locate turtle nests, but Burke et al. (2005) found that human scent around turtle nests decreased predation. Therefore, researchers must design nest census programs and experiments so that they can assess the role that researchers play, if any, in the discovery of nests by predators. Such information is especially important when implementing research and management plans for declining populations of turtles (e.g., Marchand and Litvaitis 2004).

Whatever proximate visual or olfactory cues predators use to locate and destroy turtle nests, the result is lower reproductive success of turtles. Other factors have more subtle or indirect effects on rates of nest predation. Predators often focused foraging along habitat edges so that predation on turtle nests was greater near habitat edges, either natural or anthropogenic (Temple 1987). Anthropogenic habitat fragmentation may create an ecological trap (e.g., Schlaepfer et al. 2002) in which the areas with the best cues for nesting are also areas of high predator activity (e.g., Shipley et al. 2013). However, consistent edge effects on turtle nest predation is not observed in all studies (Kolbe and Janzen 2002a), and selection of nesting location in relation to edge is influenced by more than predation rates (Spencer and Thompson 2003). Indeed, Strickland and Janzen (2010) found reduced turtle nest predation near anthropogenic structures. Predators may also time searches for nests during periods of peak laying.

In this study, we used artificial turtle nests to examine the cues used by predators to locate turtle nests, including the impact of marking flags, disturbed soil from excavation, and the presence or absence of an egg. We also assess whether predation rate varies with distance from habitat edges. Previous experiments that used artificial nests were done at sites in which all natural turtle nests were made by semi-aquatic or aquatic turtles. Unlike those studies, the most common nesting turtle at our study site was a terrestrial species, the Ornate Box Turtle (*Terrapene ornata*). Our experiments allow us to determine: (1) what cues predators use to find nests in an area used by nesting Ornate Box Turtles; (2) whether distance from woody-prairie edge affects nest predation rate; (3) whether flagging contributed to predation of nests; and (4) timing of nest predation relative to establishment of artificial nests.

Materials and Methods

We studied Ornate Box Turtles within an area of approximately 75 ha in the Hawkeye Wildlife Area (HWA), Johnson County, Iowa (Fig. 1). The HWA, owned by the U.S. Army Corps of Engineers and managed by the Iowa Department of Natural Resources, is located on the south side of the Iowa River. A series of sand dunes in HWA contains the largest concentration of Ornate Box Turtles. The study area adjoins relatively undisturbed habitat to the north and farmland to much of the south, east, and west.

We focused this study on two nesting areas (Mallard Pools and Greencastle) separated by approximately 1.3 km with no apparent barriers to migration (Fig. 1). The Mallard Pool site centered on a relict elevated dune covered with sand prairie vegetation. The dune was undergoing woody succession and was flanked on most sides by a mixture of blackberry species (*Rubus* sp.), Prickly Ash (*Zanthoxylum americanum*), Rough-leaved Dogwood (*Cornus drummondii*), and other woody shrubs. The sand prairie vegetation was dominated by a variety of forbs and grasses, and the northwest corner of the dune contained a planted grove of Pines (*Pinus* sp.) and a wetland (Fig. 1). The dune at Greencastle was dominated by planted tallgrass prairie species such as Big Bluestem (*Andropogon gerardii*) and Little Bluestem (*Schizachyrium scoparium*). The habitat surrounding the dune at Greencastle consisted of Smooth Sumac (*Rhus glabra*), planted pines, a planted grove of Catalpa (*Catalpa bignonioides*), and narrow bands of hardwoods flanking wetlands. Red Cedars (*Juniperus virginiana*) were invading both areas but were more numerous and taller at Greencastle. Our past studies (Neil Bernstein, unpubl. data) indicated most nests were depredated within 24–48 h of laying at Mallard Pools and Greencastle, and nests were clustered in space at Mallard Pools.

During June, July, and August 2008, we established eight transects at Mallard Pools and five transects defined at Greencastle. At Mallard Pools, each transect extended from a shrub edge into the sand prairie and contained three sets of experimental arrays at 5, 15, and 25 m from the edge. At Greencastle, each transect extended from a forest edge into the sand prairie and contained two sets of experimental arrays at 5 and 15 m from the edge. The transects did not extend beyond 15 m at Greencastle because the open prairie habitat was narrower than at the other sites. Past studies (Bernstein et al. 2007) indicated that Mallard Pools and Greencastle were primary nesting areas for Ornate Box Turtles as well as locations where the turtles spent most of the year (Bernstein and Black 2005).

To assess cues predators use to detect turtle nests, each experimental array consisted of four artificial nest treatments: (1) Disturbed Soil/Flag (soil disturbed with a hole excavated to about 15 cm deep and refilled with soil to simulate a box turtle nest with a 0.5 m wire surveyor's flag); (2) Egg/Flag (a buried Chicken, *Gallus gallus*, egg marked with a flag); (3) Egg/No Flag (a buried chicken egg with no flag); and (4) Flag Only (undisturbed soil/no hole excavated marked with a flag). A predator approaching the plot has an array of independent, discrete choices. Each experimental array had up to four, independent choices, and the experimental stations were also independent of each other. We placed flags within 1 cm of the treatment.

Treatments were arrayed at the corners of a 1 × 1 m square, and we randomized the placement of the treatments at each array at the start of each month. We moved transects 5–10 m at the beginning of each month so that the experiments were not in the exact same location between months, and treatments were re-randomized. We monitored each site for digging and/or depredation of the eggs (nests) every morning for the next 24 d. We removed egg shells found after depredation.

To accommodate the spatial arrangement of the treatments in the square, we chose one treatment, Egg/Flag, as the baseline against which to compare the others. We considered the Egg/Flag treatment to be the most extreme treatment (chicken egg present, disturbed, and flag) so the expectation was that Egg/Flag would be the treatment that would be depredated first (i.e., have the shortest survival time). The response is Difference in Duration defined to be duration (treatment j)-duration (Egg/Flag). We used a linear model of Site-Month, transects nested within Site-Month, distance from edge, and treatment. Under the null hypothesis, the Difference in Duration variable is

Figure 1. Hawkeye Wildlife Area, Johnson County, Iowa, showing locations of Mallard Pools and Greencastle study areas. (Photograph from Iowa Geographic Map Server: Iowa State University Geographic Information Systems Support & Research Facility, Ames, Iowa).

symmetric around zero so the Gaussian distribution was used for the error structure. We assessed significance with $\alpha = 0.05$, and we calculated 95% Confidence Intervals for the response by treatment. We analyzed data using R version 2.15 (The R Foundation,http://www.r-project.org/).

Results

Predation differed significantly among treatments ($F_{2,238} = 975.8$; $P < 0.001$). For the 24 d period, Flag Only (undisturbed soil) averaged 23.1d survival, an average of 20.6 d longer than the buried egg marked with a flag (Table 1). In contrast, the average differences in survival between the buried egg and other treatments were only between 0.12–0.60 d (Table 1). On average, any treatment with disturbed soil was depredated within 2–3 d (Table 1). There were no significant differences in nest survival among site-month, transect, and distance from edge (all $P > 0.20$, all tests). The July survival of the four treatments over 24 d at Mallard Pools is representative of our observations (Fig. 2).

Indicative of how quickly a nest can be depredated, during the time of the experiment, a partially eaten female was depredated while laying eggs. Her right rear leg was missing with the viscera exposed, and her right front leg was mostly gone. Two eggs were eaten outside the nest, and two unlaid eggs were found inside her oviducts.

Discussion

Predation rates on artificial nests with disturbed soil were uniformly high, many within 48 h. This is consistent with our observations of natural nests, which are heavily depredated in the first

Table 1. Mean monthly treatment survival of experimental nests (days) and mean difference between Egg/Flag and all other treatments (Disturbed Soil/Flag, Egg/NoFlag/ Flag Only). For all treatments, n = 92; SE = standard error; CI = confidence interval.

Treatment	Mean Duration (SE)	95% CI	Mean Survival (SE)	95% CI
Egg/Flag	2.5 (0.31)	1.85–3.07	–	–
Disturbed Soil/Flag	2.6 (0.36)	2.58–3.29	0.1 (0.27)	-0.41–0.66
Egg/No Flag	3.1 (0.49)	2.07–4.04	0.6 (0.36)	-0.12–1.31
Flag Only	23.1 (0.35)	22.36–23.77	20.6 (0.45)	19.71–21.51

few days after oviposition, but virtually never after two weeks. Natural nests at Mallard Pools were clustered in space and time near the crest of the dune in sparsely vegetated sand (Neil Bernstein, unpubl. data). Even when not nesting, adult turtles in this population concentrate their activity on and around the sand dune, which is surrounded by woody vegetation; females make nests and overwinter on the dune within a meter of previous years' locations (Bernstein et al. 2007). Because nesting occurs over a period of only about two weeks and nests are clustered in a small area, predators can readily locate new nests. Predator activity is also concentrated around the nesting periods in the Painted Turtle (Snow 1982; Christens and Bider 1987; Wirsing et al. 2012), Diamondback Terrapin (*Malaclemys terrapin*; Butler et al. 2004), and Snapping Turtle (*Chelydra serpentina*; Congdon et al. 1987; Robinson and Bider 1988; Wirsing et al. 2012), and excavation of turtle nests shortly after laying have been reported in many studies (e.g., Kolbe and Janzen 2002a, b; Marchand et al. 2002; Burke et al. 2005; Schneider et al. 2011; Wirsing et al. 2012).

Neither flags nor eggs significantly affected predation rates. As noted, there is much speculation concerning how predators locate turtle nests and whether methods used to mark natural and artificial nests affect predation. While mammalian predators potentially use scent of the egg to find nests, there was no difference in predation rates of artificial nests with and without eggs. It is also logical to assume that predators could learn to search for nests that are flagged, however, nests containing eggs were excavated at the same rate with or without a flag as a marker. The only treatments that survived in our study were those with flags but no soil disturbance. These results agree with Burke et al. (2005) who found flagging did not increase nest predation, and like Strickland et al. (2010), soil disturbance was a major cue predators used to find nests in our study. However, if predators detect disturbed soil by scent, olfactory cues could be important stimuli to locate turtle nests also.

Whereas habitat fragmentation is a threat to turtle species (Mitchell and Klemens 2000), we found no evidence to support increased predation rates on Ornate Box Turtle nests near edges as documented by Temple (1987) for natural nests. However, our definition of edge was more restricted than that of Temple (1987) who defined edge as <50 m from a marsh or forest border, and none of our nests, natural or artificial, were >50 m away from a woody edge. However, our results regarding edge or proximity to woody vegetation agree with a study on Painted Turtles (Kolbe and Janzen 2002a), who also found nests clustered in sites with less vegetation and more sand. In our study, predation on artificial nests was independent of distance from the woody edge and occurred within days.

In contrast, Marchand et al. (2002) studied artificial nests at varying distances from the edges of ponds, and, whereas clustered nests were more subject to predation, nests >50 m from the pond edge were destroyed less often by predators.

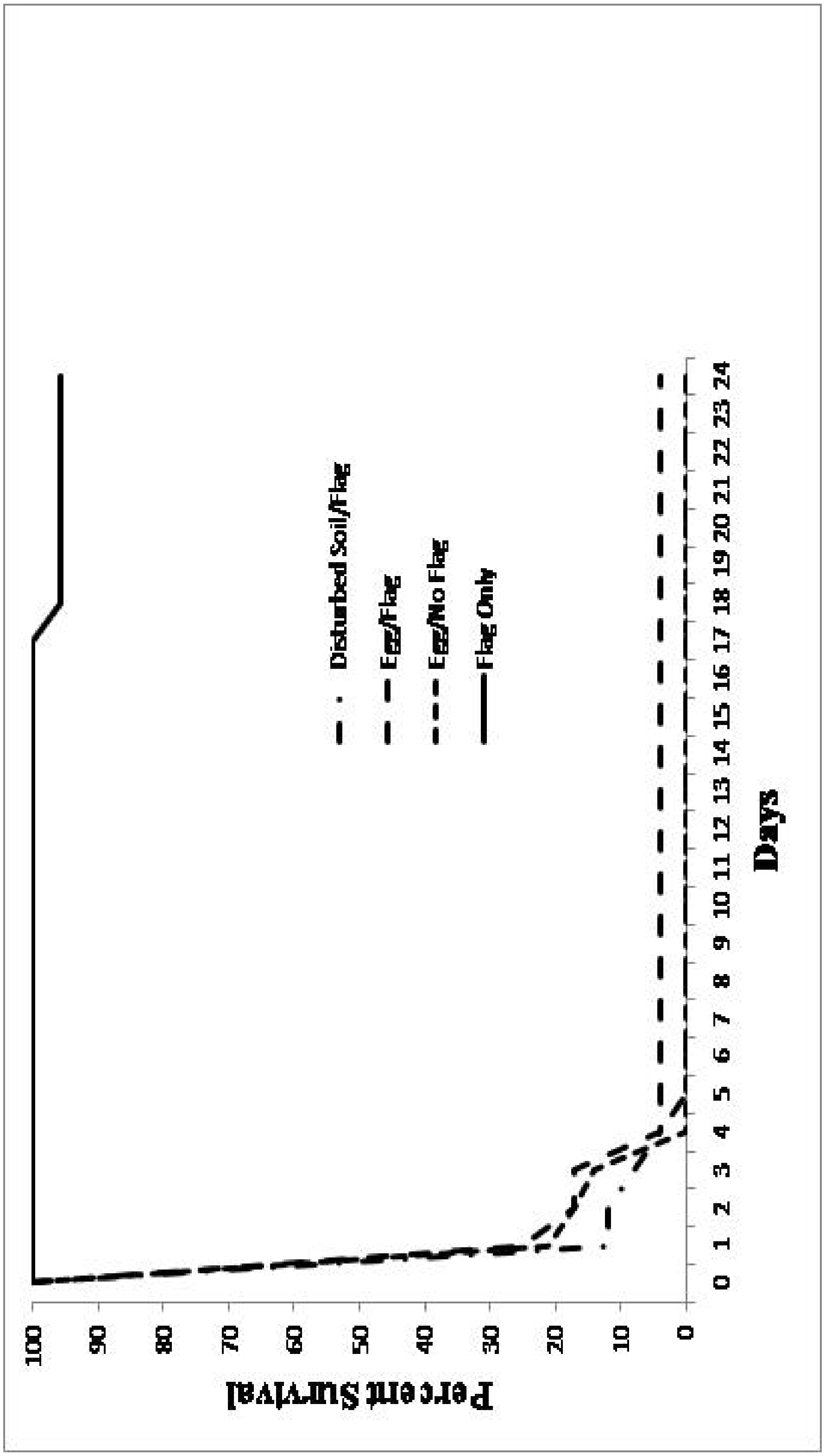

Figure 2. Mean daily percentage survival at Mallard Pools in July as an example of typical survival patterns of experimental nests. Disturbed Soil/Flag, Egg/Flag, Egg/No Flag, and Flag Only.

Therefore, predators of aquatic turtle species may focus on where females emerge to nest, and studies documenting mesopredator activity in woody edges (Pedlar et al. 1997; Dijak and Thompson 2000; Kuehl and Clark 2002) may not be predictive of aquatic turtle nest success.

Congello (1978), Bowen and Janzen (2005), and others have noted an increase in turtle nesting during nights of heavy rainfall. We also observed increased nesting activity during nights of rain in our study (Neil Bernstein, unpubl. data), and whereas heavy rainfall may indeed reduce scent of the nest, rainfall also obscures excavation in the soft sandy soil used by nesting Ornate Box Turtles in our study, thereby decreasing some stimuli associated with soil disturbance. From an evolutionary and ecological perspective, nesting in sandy open areas during nights of heavy rainfall is in agreement with the strategy of selecting oviposition sites to maximize embryo survival (Refsnider and Janzen 2010).

Therefore, for artificial nests constructed in Ornate Box Turtle nesting areas, we found no evidence that egg scent or flagging were sole cues predators used to find nests despite high rates of predation within 48 h of initiation of the experiments. Human scent also did not seem to matter as all experimental treatments were within 1 m of each other and visited daily, and only undisturbed soil did not elicit digging. At least on our site, excavation of nests did not differ within 25 m of the woody edge, and all experimental nests except the flag only (no excavation) were quickly located by predators. However, we cannot rule out that scent of disturbed soil might be a stimulus predators use to locate nests.

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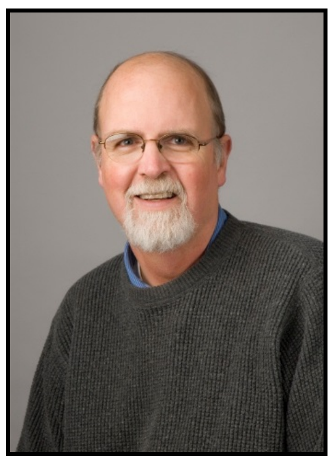

Robert W. Black earned a Ph.D. from State University of New York Stoneybrook and began teaching at Cornell College in 1987. He and his students began researching Ornate Box Turtles in 1996, and he also developed student-centered research on Iowa raptor migration and fire coral ecology in the Bahamas. Dr. Black was well-known for his love of students, the outdoors, and athletics, and he volunteered as an assistant basketball coach at Cornell. He always encouraged students to explore and cherish nature and wilderness. Dr. Black retired from Cornell in spring 2010 after being diagnosed with ALS. He passed away on 13 January 2012, shortly after beginning to work on this manuscript. (Photograph from Cornell College).