

**Research Article**

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The Effects of Plastic and Metal Predator Excluders on Diamondback Terrapin (*Malaclemys Terrapin*) Nest Temperatures, Hatching Success, and Proxies of Hatchling Sex

Russell L Burke^{1,2*} and Adriana M Eugene¹¹ Department of Biology, Hofstra University, Hempstead, NY, 11549, USA² CUNY Graduate School of Public Health & Health Policy, 55 West 125th Street, New York, NY 10027***Corresponding author:** Russell L Burke, Department of Biology, Hofstra University, Hempstead, NY, 11549, USA biorlb@hofstra.edu**Received Date:** April 01, 2024**Published Date:** April 26, 2024**Abstract**

Turtles are among the most threatened vertebrate groups, and conservation efforts to protect turtle populations commonly include the use of predator excluders to protect nests. There is a wide diversity of excluder designs and methodologies, and their potential effects on incubation conditions are little explored. Conservation efforts could benefit from analyses indicating which excluder design has the least effect on incubation conditions, and thus how to minimize effects on hatchling success, sex ratios and hatchling survivorship. We tested the effects of two commonly used predator excluder designs on Diamondback Terrapin (*Malaclemys terrapin*) nests in Jamaica Bay, New York. We measured nest temperatures throughout the incubation period and focused on the temperature sensitive period, when hatchling sex is determined. We found that the predator excluders had no effect on the temperature at which the nest were incubated or hatching success, and thus can be used with confidence.

Keywords: Conservation; nest protection; turtle**Introduction**

Turtles (order Testudines) are among the most endangered vertebrate groups; 10% of turtle species are critically endangered and many more are threatened [1,2]. Predation on turtle nests has had a detrimental effect on marine and freshwater turtle populations globally, and in many cases is one of the most important detriments to conservation efforts. In particular, populations of human-subsided predators have increased in many locations in recent years due to human activity, through increases in food resources,

habitat changes, and meso-predator release [3]. In North America, human-subsided predators such as raccoons (*Procyon lotor*) are often the most important predators of turtle nests [4], however a wide array of other species depredate turtle nests globally. Conservation programs often include protecting turtle nests from predators via the use of predator excluders; these can reduce predation rates from 100% predation to near 0% [5,6]. Such excluders are sometimes used in situ, other times excluders are used to protect

relocated nests. Excluders are typically constructed from fencing material, but materials, designs, and placement methodology vary among conservation programs. Metal wire mesh excluders are common, and different types have been described [7-13].

Diamondback Terrapins (*Malaclemys terrapin*) are a common focus of conservation programs that use predator excluders at many sites throughout their range along the eastern and Gulf coast of the United States from Massachusetts to the Texas-Mexico border (Burke, pers. obs.). Diamondback Terrapins are listed as "Vulnerable" on the IUCN Red List of Threatened Species [14] and are protected by state regulations in all 16 states in their range [15]. Nest predation by a diverse suite of predators dramatically decreases terrapin nest survival [16-18]. The relationships between Diamondback Terrapins and nest depths, nest temperatures, and hatching success are complicated; deep nest are cooler and have higher hatching success in unusually warm, dry summers, while the reverse is true in cool, damp summers [19]. Diamondback Terrapins also have temperature sex determination Type IA, in which at high incubation temperatures produce female and low incubation temperatures produce male hatchlings [20]. The temperature sensitive period (TSP) spans incubation days 28-35; this is the period during which sex is determined in the developing embryos [20]. We sought to test the generality of Riley and Litzgus [5]'s findings by testing predator excluders on a new turtle species (*M. terrapin*) at a lower latitude that is a more typical climate regime for most North American turtles. We tested a metal wire mesh excluder design as did Riley and Litzgus, and also a plastic mesh excluder design, which we hypothesized might have very different thermal characteristics than the wooden or metal designs they tested. We also focused on the possible effects on hatchling sex determination that excluders might have.

Methods

We conducted our experiment on Ruler's Bar Hassock (RBH), the central island in Jamaica Bay, Queens, New York. Diamondback Terrapin nesting ecology has been studied at this site since 1998 [16, 21-26]; 700-2000 Diamondback Terrapin nests were laid on RBH annually, and raccoons are the only important Diamondback Terrapin nest predators [16]. Approximately 67-98% of unprotected nests were predated by raccoons during 1999-2018 [16,26]. RBH had a density of 0.27 to 0.61 raccoons/ha [27]. Our experiment was conducted in open, sandy soil habitat in areas with high terrapin nesting density during 1 June-31 July 2016 during the normal Diamondback Terrapin nesting season at RBH. We located nesting Diamondback Terrapins by searching known nesting areas many times daily. As each Diamondback Terrapin completed nesting we marked the nest site and returned later the same day to excavate the nest, count and measure eggs, and measure nest depth. In some cases, we re-buried eggs in the original nest cavity, in other cases, we constructed new nest cavities because the original nests were deposited where they would be vulnerable to vehicular and pedestrian traffic. Moved nests were reconstructed within 24 hours of

oviposition and within 3 meters of original nest sites, in substrates and areas commonly used by other nesting Diamondback Terrapins, and both the nest cavity shapes and depths were excavated so as to be similar to the original nests.

We set Model DS1922T, iButton temperature loggers to record temperature hourly for approximately 80 days and buried them in the middle of each clutch when it was reburied. We waterproofed loggers using black Plasti Dip; Roznik and Alford [28] showed that this treatment does not affect the ability of iButtons to accurately record temperature data. As each set of three terrapin nests became available, we randomly assigned one nest to a metal nest protector treatment, then we randomly assigned one nest to plastic nest protector treatment, and the remaining nest was assigned to a screened control treatment (see below). We protected each nest with one of two predator excluder designs. One design was metal wire mesh (1.27 cm square-holed hardware cloth, cube-shaped excluder 30.5 cm sides and height, Figure 1); this is the primary excluder design that has been used on RBH projects for the last decade, has been generally successful at excluding raccoon predation, and is similar to the above-ground design used by Riley and Litzgus [5]. As has been done previously at RBH, we implanted the sides of the metal excluders 10-15 cm into the ground and implanted 4-6 metal stakes in the ground through the screening distributed around each excluder to anchor the excluder and discourage predators from digging underneath.

The other predator excluder design was made with black 1.27 cm square-holed Tenax PVC Hardware Net (Figure 2). This design has been used in a Diamondback Terrapin conservation project conducted by the Wetlands Institute (Stone Harbor, NJ) in cooperation with the Richard Stockton College (Galloway, NJ) since 1989. These excluders were cylindrical, open at one end, 24.5 cm high and 20.23 cm in diameter, and secured in shape with zip ties. We secured a square piece of the same plastic material over the top of the cylinder with zip ties. We employed these excluders by burying the sides 5 cm deep. We used garden stakes to anchor each plastic excluder by pushing the stakes into the ground through the plastic mesh and into the ground; we distributed the stakes around each excluder. We paired excluder-nest combinations in arrays with two types of controls to simulate conditions without excluders. We designed one set of controls (screen controls) to account for the unknown but potentially relevant amount of metabolic heat given off by Diamondback Terrapin eggs as they incubate. For each of these controls we constructed a Diamondback Terrapin nest near (within 2 meters) of an excluder-nest combination; each of these control nests had a full nest (as originally laid) of Diamondback Terrapin eggs and a temperature logger as described above. Instead of an above-ground excluder, we covered these controls with a flat horizontal metal screen to dissuade predation (constructed of 1.27 cm square-holed hardware cloth, square-shaped, 30.5cm sides, anchored at each corner with a metal stake) and covered with local substrate to eliminate solar exposure.



Figure 1: Metal wire mesh predator excluder.



Figure 2: Plastic (Tenax PVC Hardware Net) predator excluder.

Our second control type (screenless control) was designed to account for the normal temperature underground at each nest site and ignoring the potential for significant amounts of metabolic heat. We constructed these just as the screen controls above, except with no screen and no eggs. As a result, each array consisted of one nest with a metal predator excluder, one nest with a plastic predator excluder, one screen control with a clutch of eggs, and one no-screen control with no eggs. We removed the flat metal screens (screen controls) August 12, 2016 in anticipation of hatchling emergence and placed plastic rings around each nest site to restrain emerging hatchlings. Burlap fabric was placed over each ring to prevent new-

ly emerged hatchlings from desiccating. We checked nests daily for hatchling emergence. After emergence, we counted the number of hatchlings that emerged from each clutch and recovered temperature loggers. We calculated hatching success as the percentage of eggs in each nest that hatched. After normality tests, we conducted one-way ANOVAs to test whether the different excluder types affected incubation durations (number of days from oviposition to first emergence), mean carapace lengths, mean temperatures over the incubation period (all hourly readings from oviposition to first emergence), mean temperatures during the TSP (incubation days 28-35, Burke and Calichio [20]), and the degree hours above devel-

omponential zero (14oC, calculated from data from Burke and Calichio [20]). Hatching success (% of eggs that hatched and emerged) was non-normally distributed; we analyzed this using a Kruskal–Wallis test.

Results

We constructed 30 arrays (30 Terrapin nests with metal excluders, 30 Terrapin nests with plastic excluders, 30 screen controls with Terrapin clutches, and 30 screenless controls with no eggs). Many arrays were disturbed by raccoons, construction and maintenance workers, and park visitors such that we either failed to recover temperature loggers, failed to collect data from hatchlings, or both. As a result, we recovered 81 (67.5% overall) of the 120 temperature loggers: 14 (46.7%) from metal excluders, 16 (53.3%) from plastic excluders, 29 (96.7%) from screen controls and 22 (73.3%) from screenless controls. We collected hatchling data from 52 (57.8% overall) of the 90 nests with eggs: 12 (40.0%) from metal excluders, 14 (46.7%) from plastic excluders, and 26 (86.7%) from screen controls. All data except hatching success were normally distributed (e.g., Levene's tests indicated equal variances, residual plots and Q-Q plots indicated no irregularities). ANOVAs indicated that there were no differences among plastic predator excluders (\bar{x} = 60.14 days, std dev = 5.77, min = 52, max = 71), metal predator excluders (\bar{x} = 59.71 days, std dev = 7.51, min = 48, max = 72), or screen controls (\bar{x} = 59.93 days, std dev = 6.06, min = 53, max = 73) for mean incubation duration ($F(2,53)=0.02$, $P=0.98$), mean carapace length (plastic predator excluders \bar{x} = 25.36 mm, std dev = 1.72, min = 21.00, max = 28.50, metal predator excluders \bar{x} = 25.36 mm, std dev = 1.35, min = 22.67, max = 27.58), or screen controls \bar{x} = 25.38 mm, std dev = 1.00, min = 23.30, max = 26.65, $F(2,47)=0.03$, $P=0.97$), or mean nest temperature during the entire incubation period (plastic predator excluders \bar{x} = 28.18oC, std dev = 0.71, min = 26.71, max = 29.12, metal predator excluders \bar{x} = 28.07oC, std dev = 0.67, min = 26.86, max = 29.51), screen controls \bar{x} = 28.53oC, std dev = 0.93, min = 26.78, max = 29.75, or screenless controls \bar{x} = 28.64oC, std dev = 1.08, min = 26.55, max = 30.88, $F(2,47)=1.61$, $P=0.21$). ANOVAs also indicated that there were no differences in mean temperature during the TSP among plastic predator excluders (\bar{x} = 28.07oC, std dev = 1.25, min = 26.46, max = 30.51, metal predator excluders (\bar{x} = 28.87oC, std dev 1.06, min = 26.59, max = 30.72), screen controls (\bar{x} = 28.75oC, std dev 1.08, min = 27.39, max = 31.06) or screenless controls (\bar{x} = 28.95oC, std dev = 1.35, min = 26.61, max = 32.04)($F(3,77)=1.66$, $P=0.18$), or mean degree hours above developmental zero (plastic predator excluders \bar{x} = 2706.20 hours, std dev = 249.74, min = 2393.5, max = 3171.1, metal predator excluders \bar{x} = 2890.91 hours, std dev = 246.69, min = 2418.4, max = 3487.4), screen controls \bar{x} = 2830.74 hours, std dev = 215.78, min = 2451.8, max = 3275.2, or screenless controls \bar{x} = 2931.08 hours, std dev = 335.15, min = 2421.1, max = 3859.5, ($F(3, 77)=2.41$, $P=0.07$). The Kruskal–Wallis test indicated that there were no significant differences among plastic predator excluders (median = 72.86, ICR = 32.5), metal predator excluders (median = 80.00, ICR = 28.0), or screen controls for hatching success (median = 90.00, ICR = 28.17, $H=5.02$, $P=0.081$).

Discussion

Predator excluders may harm incubating turtles if the excluders alter incubation conditions, which they might do either by shading nests (cooling) or by trapping heat near the nest (greenhouse effect). Nest temperature is important because incubation temperature affects many characteristics of turtle eggs and resulting hatchlings, such as hatching success rates, incubation duration, body size, yolk reserve, and locomotor performance [29-33]. Incubation temperature can also influence hatchling sex in many turtle species [34]. Furthermore, excluders could alter hydric conditions in nests by deflecting rainfall, by altering temperature that determines drying rates, or by functioning as a solar still, condensing and returning evaporated substrate moisture. Nest substrate moisture can affect hatchling size, hatching success, incubation duration, and hatchling size [35-37]. Nest temperature and moisture can have synergistic effects because moisture can lower nest temperatures enough to alter hatchling sex ratios [38-40].

Riley and Litzgus [5] tested the effects (nest temperatures, hatching success, frequency of hatchling deformities, morphology and locomotor performance) of wooden-sided, above ground and below ground wire predator excluders on the nests of Painted Turtles (*Chrysemys picta*) and Snapping Turtles (*Chelydra serpentina*) near their northern range limits. They found that the excluder designs they tested only affected hatchling body condition (mass/carapace length), and not nest temperatures, frequency or morphology of hatchling deformities, or locomotor performance. We found that two predator excluder designs, one made of metal and one of plastic, both of which have been effective at reducing raccoon predation on Diamondback Terrapin nests, also had no detectable effect on incubation temperature, hatching success, or hatchling carapace length. We further suggest that these designs had no effect on hatchling sex ratios, based on their lack of effect on our indirect measures of sex determination (mean temperatures during the temperature sensitive period, mean degree hours above developmental zero, incubation duration). Thus, our results concur generally with those of Riley and Litzgus [5], indicating that common predator excluder designs do not appear to adversely affect nest incubation conditions.

We note that hatching success rates we measured for metal screened nests were highly variable and that some of our p values for ANOVAs were low, and being aware of critiques of application of absolute alpha levels [41], we calculated minimum sample sizes necessary to detect significant differences using our data as a pilot study using Ristl (2021) [6]. We found that 3997 replicates/treatment would be necessary to detect significant differences among mean incubation duration, 75,964 replicates/treatment to detect significant differences among mean carapace lengths, but only 42 replicates/treatment for mean temperature during the entire incubation period, 36 replicates/treatment for mean temperature during the TSP, 32 replicates/treatment for mean degree hours above developmental zero, and 43 replicates/treatment for hatching success (calculated by adding 15% to parametric results, as recommended by Lehmann [42]). Given these results, we strong-

ly suggest that other researchers/conservationists conduct similar studies to confirm the results for the species in which they are studying, especially if the species is larger in size, lays bigger clutches, is in a different climate zone, uses different nesting substrate, or if they use different excluder designs or materials.

Some variations in excluder materials and design are probably due to different nest sizes, substrate types, cost, and availability of available construction materials, but we suspect many aspects of predator excluder designs are determined mostly by local tradition, and effectiveness at thwarting predation is rarely evaluated. Bougie et al. [43] found that excluders varied in efficacy during incubation and between years. Campbell et al. [44] developed and tested a relatively rapid methodology to assess the effectiveness of turtle nest protector programs. Nordberg et al. [45] found that the effectiveness of sea turtle nest protectors varied by predator species. For example, Lamarre-DeJesus and Griffin [46] found that applications of habanero pepper (*Capsicum chinense*) on freshly oviposited loggerhead sea turtle (*Caretta caretta*) nests where coyotes (*Canis latrans*) were the main nest predators had 2.5 times higher survival than did control nests, whereas similar applications on Diamondback Terrapin nests did not reduce predation by raccoons [24]. Buzuleciu et al. [47] reported three novel excluder designs, one using galvanized steel ducting, one using plastic mesh, and another using metal wire mesh. Quinn et al. [48] described the use of a nest box with electrified wiring and covering an artificially constructed nest mound that dramatically reduced predation rates.

We found that both metal and plastic mesh materials were equally effective in deterring raccoon predation, and the excluders made from plastic mesh were easier, less expensive, and safer to make. Volunteers were able to produce plastic excluders in about half the time required to make metal excluders. However, plastic excluders may be less effective at deterring larger or more persistent predators than raccoons and may not be as effective at other sites. Bougie et al. [43] recommended that turtle nest protectors be tested in different areas with different suites of predators, because their efficacy may vary regionally, and we concur. The protocols we used here could easily be replicated in turtle conservation programs elsewhere.

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