NORTHEAST PARTNERS IN AMPHIBIAN AND REPTILE CONSERVATION

2021 Annual Meeting – August 11 to August 12

Virtual

ORAL PRESENTATION ABSTRACTS

(in order of presentation)

Title: The Collaborative to Combat the Illegal Trade in Turtles: Addressing a Critical Conservation Threat with a Call to Action

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Abstract: The illegal collection and trade of North America's native turtles is a serious threat with potentially severe conservation implications. The challenges this issue raises are variable and complex and confronting them will require creating a professional network of law enforcement personnel, biologists, legal experts, and others with expertise in husbandry, economics, and human dimensions. In response to this need, the Collaborative to Combat the Illegal Trade in Turtles (CCITT) was established in 2018. The CCITT is a grassroots group whose goal is to advance efforts to better understand, prevent, and eliminate the illegal collection of North America's native turtles. The group has grown quickly in under 3 years and currently consists of over 90 members, composed primarily of biologists and law enforcement officers from state and federal agencies, but with members from academia and NGOs as well. The geographic footprint of membership continues to grow as well and the intent of the group is to address needs and develop tools and resources that can be applied nationwide. Many of these needs were identified in a Call to Action Letter to Protect North America's Turtles from Illegal Collection released in 2020. In the letter, CCITT along with Partners for Amphibian and Reptile Conservation, the Association of Zoos and Aquariums, and the Wildlife Trafficking Alliance, outlined a set of priority actions for establishing a united front against criminals who are trafficking in the nation's natural heritage. Goals outlined in the letter and current efforts underway will be discussed.

Title: Informing Spotted Turtle (*Clemmys guttata*) Conservation Ecology and Management on Department of Defense Installations

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Abstract: The Spotted Turtle (*Clemmys guttata*) is a small, semi-aquatic freshwater turtle that ranges from Maine to Florida in the Eastern United States, with disjunct populations in the Midwest. The Spotted Turtle has been petitioned for federal listing under the Endangered Species Act because populations across the range are threatened by habitat loss, climate change, and illegal collection. In 2018, the Spotted Turtle Working Group (STWG) began conducting a status assessment from Maine to Florida to develop a regional conservation plan. The DoD owns or manages nearly 25 million acres of land in the U.S. for its military operations and has the greatest density of imperial species than any other federal agency. Spotted Turtles are known to occur on 39 installations and potentially occur on an additional 60. These installations will be essential for sustaining populations as habitat loss and climate change continue to degrade habitat suitability. To capitalize on the expertise of the STWG and address the need on installations, the Department of Defense's Partners in Reptile and Amphibian Conservation (DoD PARC) partnered with members of the STWG to assess Spotted Turtle distribution and abundance on nine military installations from Massachusetts to Georgia. From March to June 2020, we identified 177 individuals among 211 captures in ~117 visual survey hours and 2,158 trap nights. This process and these data will inform the regional status assessment and provide a replicable model for conservation of Spotted Turtles and other imperiled species on DoD installations.

Title: Cricket Frog Extirpation in the United States Based on Historical Records from 1960-2020

Graduate Student Author(s): Elise M. Edwards*, Cornell University, Natural Resources Department, Ithaca, NY 14850; eme42@cornell.edu

Abstract: Cricket frogs (*Acris spp.*) are distributed widely throughout the eastern and central United States. However, cricket frogs have been experiencing drastic declines in the northern part of their range for the past 30-50 years. Declines were first reported in Ontario, Canada with the species considered extirpated in 1987. In the United States, local declines and extirpations have been documented in Colorado, South Dakota, West Virginia, Arizona, Iowa, Illinois, Minnesota, Iowa, Michigan, Indiana, Wisconsin, Ohio, Pennsylvania, and New

York. A wide range of studies have looked at causes of localized cricket frog decline, including multi-state scale approaches, many of which have come to a wide range of conclusions about the changes of the cricket frog population status. The cricket frog poses a fascinating and informative case study because it indicates that the disappearance of this amphibian species does not occur throughout its range, but only at the northern extent. Previously, studies have taken a localized approach at determining the reasons for cricket frog decline and this problem needs to be examined across the entire species range. We examine broad scale trends of cricket frog decline by looking at historical survey data in the context of climatic and atmospheric change as well as a broad scale land use change to better assess what factors may have led to cricket frog decline at a broad scale. This work presents a historical record of cricket frog extirpation and will include possible reasons of extirpation related to extreme overwintering temperatures, urbanization, and habitat fragmentation.

Title: Development of a Skin Probiotic to Mitigate Chytridiomycosis in Eastern Newts in Massachusetts

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Abstract: Chytridiomycosis is a disease caused by fungal pathogens *Batrachochytrium dendrobatidis* (Bd) and *B. salamandrivorans* (Bsal), which have been implicated in global amphibian population declines. Although Bsal has not yet been detected on North American amphibians, lab trials indicate susceptibility to fatal disease in the geographically widespread Eastern Newt (*Notophthalmus viridescens*), and stream salamanders in the genus *Eurycea*. Disease management tools under development include fungicides, a vaccine, and bioaugmentation. Toward the aim of proactively treating amphibians with protective probiotic bacteria that can persist on newt skin and be transmitted within natural amphibian assemblages, we developed a strategy for developing a safe and effective probiotic treatment. Thus, we cultured skin-associated microbes from native Eastern Newts and tested their ability to inhibit the growth of both Bsal, and the endemic pathogen Bd. Several isolates inhibited both pathogens. Various non-target organisms including *Daphnia, Physa* snails, *Ambystoma* salamander larvae, mosquitoes and fish are being exposed to a range of doses to assess ecological safety of the probiotics in lab and mesocosm settings. Upon determination of effective applications of probiotics to newts that reduce Bsal and Bd infections, we will use control and experimental pond sites to assess the effectiveness of probiotic application at reducing Bd pathogen loads on wild Eastern Newts. Persistence and transmission of the probiotic will be monitored to determine whether reapplication is necessary to proactively boost the immunity of Eastern Newts against invading chytrid pathogens.

Title: American Turtle SAFE: Building the Path from Confiscation to Conservation in Real Time

Author(s): Dave Collins*, Turtle Survival Alliance, Charleston, SC 29407; dcollins.glin@outlook.com

Abstract: Addressing the immediate need of providing housing and care for turtles confiscated in illegal trade is of highest priority to the AZA SAFE: American Turtle Program. However, the ultimate success of the program also demands a comprehensive and forward-looking plan that will ensure the best outcome for those animals. This will require a continuum of high-quality care, often provided by several different partners. The nature of the illegal trade is so highly variable that it is essential that the plan has the flexibility and resiliency to accommodate dramatically different scenarios. Feedback on the implementation of procedures and protocols in each case provides important guidance to the continued development and improvement of the over-all plan. Case studies are a critical part of the plan. Several examples provided here illustrate how each case helps inform this process.

Title: Nest Characteristic Comparison between Developed and Enhanced Sites Selection of Diamondback terrapin (*Malaclemys terrapin)*

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Abstract: Natural populations of diamondback terrapins (*Malaclemys terrapin*) have been negatively affected by pressures from the food industry, the pet trade, road mortality, and loss of nesting habitat from human development. In many cases, natural sand-based nesting sites have been replaced with harder substrate, such as gravel. With the disappearance of natural sand-base nesting sites, nest site enhancement projects are underway for terrapins, across their range by adding sand. This study, conducted in June 2021, focused on differences between nesting ecology at terrapin nesting locations; developed (gravel-based) sites and enhanced (sand-based) sites created specifically for terrapins. Nest measurements taken at both developed and enhanced sites included nest depth, plug angle, and individual egg morphometrics. Nesting female morphometric data included age, size, and rear limb length. These parameters may help us to better quantify differences in each nest habitat type in relation to the female's morphometrics. Overall, this study should provide insight into nesting characteristics female terrapins experience due to the impact of human development. At the end of the study, each nest will be examined for hatchling emergence to determine possible effects of site characteristics on hatchling success. We hope the results of this study will shed light on the potential benefits of creating enhanced nesting locations, and aid conservation efforts to stabilize populations of diamondback terrapins.

Title: Vegetative Indicators for Habitat Preferences of Wood Turtles (*Glyptemys insculpta*) in Central Appalachian Forests

Author(s): Steven Krichbaum*, Staunton, VA 24401; Lokitoad@gmail.com

Abstract: Over four summer field seasons, vegetation was identified, measured, counted, or characterized at 400m^2 and 1m^2 plots at 640 points, evenly distributed between adult Turtle points (81 individuals) and random points, at neighboring montane study sites in the ridge-and-valley Appalachians of Virginia (VA) and West Virginia (WV) USA. Over thirty herbaceous and woody seedling taxa were indicators for Wood Turtles at these VA and WV study sites. Wood Turtle plots in both states had greater herbaceous taxa richness and greater herbaceous cover than did random plots. For both herbs and woody seedlings there were relatively few wetland taxa, with most of the taxa found in Turtle plots being "facultative" upland or upland taxa. With regard to overstory trees, Wood Turtles generally tended to prefer sites with relatively higher importance values for White Oak, Sugar Maple, Red Maple, and White Ash and relatively lower IVs for Chestnut Oak, Scarlet Oak, and White Pine. The Turtles used delineated "stands" of 10 different deciduous, mixed, or coniferous forest types. The coarse-scale stand analyses did not capture the Turtle's preferential use of habitat types. With regard to some coarse-filter structural differences (*i.e*., seral stages of stands), Wood Turtles exhibited a tendency to avoid mid-successional and early successional habitat (esh here refers to young stands of forest regenerating after intensive logging). Though often characterized as a riparian species or denizen of wet areas, in the summer these VA and WV Wood Turtles clearly use relatively closed-canopy dry uplands a great deal.

Title: Evaluating Novel Methods to Detect Bog Turtles and to Characterize Population Demography and Abundance

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Abstract: Applying effective conservation strategies for an endangered species requires a reasonable understanding of its distribution on the landscape. Significant data gaps exist in the known distribution of bog turtles (*Glyptemys muhlenbergii*) in the southern portion of the species range (VA, NC, TN, GA, and SC). Low-

density populations, the cryptic nature of the species, and financial limitations have contributed to this problem. Conventional means for detecting bog turtles (visual surveys, probing, and trapping) are logistically, temporally, and financially intensive and require a level of expertise. The limited timeframe for peak activity (May-June) also hinders the number of sites that can be evaluated annually via these methods. We are currently evaluating two novel methods (eDNA and camera traps) for detecting bog turtles in an experimental framework. This work is taking place in 12 occupied NC sites of variable turtle abundance and density. Although data are currently being analyzed, preliminary results suggest that these techniques are viable options for determining occupancy of bog turtles. Camera traps also identify high use areas and activity periods, which may help inform where and when to survey via conventional means. Excitingly, this passive technique also provides insight on relative abundance and population demography, which is invaluable for ranking populations and developing region-scale conservation plans. It appears that these techniques may greatly expand our capacity to understand bog turtle distribution and status. These techniques also hold promise for other cryptic species that live shallowly inundated wetlands such as spotted turtles (*Clemmys guttata*) and southern bog lemmings (*Synaptomys cooperi*).

Title: Minimizing Impacts of Stream Restoration Projects On Bog Turtles: Case Studies From Maryland

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Abstract: Stream restoration projects have become commonplace to meet requirements of EPA's Total Maximum Daily Load (TMDL) for sediment and nutrient discharge established in Maryland's Watershed Implementation Plan for the Chesapeake Bay. Goals of these projects include treatment of impervious areas, reductions in sediment and nutrients (e.g., nitrogen and phosphorus), raising the stream bed (incised streams), flood attenuation, stream bank stability, providing a high width to depth ratio, and increased floodplain access. Conflicts can arise when these projects intersect with wetlands occupied by bog turtles. This presentation will focus on case studies of three recent stream restoration projects in Maryland adjacent to occupied bog turtle wetlands and measures taken to decrease the likelihood of "take" of bog turtles. Telemetry and other turtle monitoring will be discussed, as well as turtle movements in relation to construction activities and potential impacts to overwintering turtles. Lessons learned and recommendations for future stream restoration projects adjacent to bog turtle wetlands, including time-of-year restrictions and other regulatory considerations, will be provided.

POSTER PRESENTATION ABSTRACTS

(alphabetical by first author last name)

Title: Larval Temperature and Salinity Conditions Influence Juvenile Wood Frog Growth

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Abstract: Vernal pool obligates, such as wood frogs (*Rana sylvatica),* face dual interacting stressors as a changing climate interacts with other anthropogenic stressors, like road salt. Our goal is to understand how combined stressors experienced by tadpoles influence juvenile wood frog growth and survival. Tadpoles were raised in a full factorial experiment manipulating temperature, with Ambient temperature conditions, Nightly (removal of nightly lows), or Spike (+6°C above ambient every third week) and salinity, with low (specific conductivity: 57-102 µS/cm) and high salinity (1900-2000 µS/cm). Frogs were placed into terrestrial enclosures at either low (2.5 frogs/m^2) or high (5 frogs/m^2) density. We surveyed enclosures every 5-10 days during active seasons, recording mass of frogs. Survival through October was comparable between enclosure densities, with 29% survival in high density, and 31% survival in low density. Growth of juvenile frogs differed between high and low density enclosures ($p = 0.0015$) as well as among frogs from different larval conditions ($p = 0.0005$). Our results support the conclusion that interacting anthropogenic disturbances during the larval life stage can affect post-metamorphic individuals. While ambient low salinity frogs grew quickest in both high and low densities, frogs from Nightly treatments grew quickly in low density but slowly in high density conditions, relative to frogs from other larval treatments. This result could indicate that ideal larval conditions prepare animals for later life stages, and fitness-reducing carry over effects may only emerge as weather patterns and vernal pool conditions shift and tadpoles experience altered larval environments.

Title: Salinity of Terrestrial Environments Effects Wood Frogs Growth in addition to Carry-over Effects from Larval Life Stage

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Abstract: Salinization of forest floors due to road salt application have reached levels that may affect biodiversity. Our goal is to understand how salinity in both aquatic and terrestrial environments affect wood frogs, including the potential carry-over effects of tadpole exposure to salinity into growth of juvenile frogs. Tadpoles were raised in a full factorial experiment that varied in water temperature (ambient and elevated +3C) and salinity (low 50-75 µS/cm, high 1900-2000 µS/cm), resulting in four larval treatments ambient temperature with low salinity (ALS), elevated temperature with low salinity (ELS), ambient temperature with high salinity (AHS), elevated temperature with high salinity (EHS). Frogs with VIE tagged and raised frogs in 5.5 gallon aquariums with sand and leaf litter. Frogs were at low density (n=4 frogs, one of each larval treatment) or at high density (n=8 frog, one of each larval treatment). In addition, we manipulated terrestrial salinity, by adding low salinity water (<200µS/cm) or high salinity water (1900-2000 µS/cm) to maintain moisture within the sand. All frogs are housed at room temperature and fed *ad libitum*. Frogs raised at low density had greater mass ($F = 13.22$, $P < 0.0003$) and at low soil salinity ($F = 5.93$, $P = 0.015$) compared frogs at high density and high soil salinity. This patterned was not consistent for all larval treatments, indicating important carry over effects on growth. This experiment should allow us to better understand how salinization of forest floors affects the growth and development of wood frogs.

Title: Thermal Preferences of Recently Metamorphosed Wood Frogs from Varying Larval Conditions

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Abstract: Predicting species response to changing climates requires an increased understanding of physiological and behavioral responses to temperature. Increasing temperatures in aquatic ecosystems alter the larval environment for ectotherms such as wood frogs (*Rana sylvatica*) which are often found to have carry over effects on frogs post metamorphosis. Our goal is to determine whether temperature in the larval environment changes thermal preferences and tolerances in the terrestrial environment in recently metamorphosed wood frogs. To investigate this question, we constructed a 150 cm long temperature gradient ranging from 15-32°C, and then observed frogs within this gradient that had been raised in four larval environments: ambient water temperature with low salinity (ALS), elevated water temperature (+3C) with low salinity (ELS), ambient temperature with high salinity of 1900-2000 µS/cm (AHS) and elevated temperature with high salinity (EHS). We observed frogs for 50 minutes using photos taken every 1 minute. We found a lot of variation among individual frogs in their choice of temperatures, and thus did not detect an effect of larval environment on frog choice of terrestrial temperatures. The large variation in temperatures that frogs used could indicate that frogs were not responding to temperature or were responding to an unknown factor, or

the length of the experiment was too short to measure the behavioral response. We will explore these alternative explanations in future experiments, such that results may eventually contribute to the understanding of amphibian physiological plasticity and acclimation to climate change.

Title: Transmission Dynamics of Ranavirus in *Lithobates sylvaticus* Juvenile Frogs

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Abstract: *Ranavirus* is a genus of double-stranded DNA viruses in the family *Iridoviridae* that infects amphibians, fish, and reptiles. Ranaviruses are responsible for mass die off events in *Lithobates sylvaticus* tadpoles and transmission readily occurs at this life stage, but little is known about transmission between recently metamorphosed frogs. This route of transmission may be very important for ranavirus epidemiology because juveniles are the life stage that move among wetlands. A continued chain of transmission between juveniles would facilitate ranaviruses being introduced into new bodies of water or repeatedly reintroduced into ponds with outbreaks. To better understand the potential for transmission between juvenile *L. sylvaticus*, we experimentally estimated the probability of ranavirus transmission in two settings. We first measured transmission between pairs of infected and naive frogs via direct contact in co-housed settings with increasing exposure durations. We then measured indirect environmental transmission from infected frogs to naïve frogs with increasing lengths of time between when the infected frog was removed and when the naïve frog was environmentally exposed. We present evidence that juvenile frogs can transmit ranavirus to naïve frogs and discuss the contexts that minimize and maximize transmission rate. The results of this research provide insight into how much direct and indirect exposure to an infected animal is necessary to cause ranavirus infection in a previously unexposed frog. Understanding the role of terrestrial interactions in the spread of ranavirus between wood frog juveniles could help explain the occurrence of ranavirus outbreaks.

Title: Mortality of Wood Frog Tadpoles Exposed to Ranavirus Dependent on Developmental Stage of Exposure and Environmental Conditions

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Abstract: Environmental conditions play an integral role in the transmission of a pathogen to host at the individual and population level. Emerging pathogens like ranaviruses are one of the leading causes of global amphibian population declines, and their lethality may be influenced by the environment in which they exist. Infections are affected by the interaction between host, pathogen, and environment via the host developmental stage, viral load, and compounding environmental stressors. For example, elevated water temperatures are known increase viral replication rates and high salinity freshwater wetlands experience more mass mortality events than low salinity counterparts. Our objective was to determine if interactions between pathogen, host development, and environmental stressors reduced survival rates of infected individuals. We raised wood frog tadpoles individually in varying environmental conditions and exposed individuals to ranavirus. Treatments included high (1900-2000 µS/cm) or low (50-75 µS/cm) salinity, ambient or elevated (ambient +3 °C) temperature. Tadpoles were exposed to ranavirus (FV3) via water bath at larval Gosner Stages 25, 30, 35 with non-exposed controls. Exposure timing and salinity reduced survival rates for virus exposed tadpoles. Survival curves unexpectedly demonstrated uniformity in timing of mortality, with deaths beginning 7-8 days after inoculation in all treatments. Notably, tadpoles exposed at stage 35 and raised in elevated temperatures and high salinity had longer cumulative survival than all other treatments with two individuals reaching metamorphosis. Our results suggest that additional research is needed to determine what larval environments may allow infected wood frogs to successfully complete metamorphosis.

Title: Evaluating the Detection of Diamond-backed Terrapin (*Malaclemys terrapin*) from an Unmanned Aerial System Using 3D Printed Models

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Abstract: Diamond-backed Terrapin (*Malaclemys terrapin;* DT) is the only brackish-adapted turtle species in North America and a species of greatest conservation need throughout most of its range. The biology of DT, including seasonal breeding aggregations, presents an exciting opportunity for revolutionizing the way wildlife populations are monitored. Unlike traditional approaches, drones offer a non-invasive and potentially unbiased sampling method with the added advantage of committing data to virtual memory. However, we know little about how drones detect DT or influence behavior; therefore, a sampling approach should determine a height detectability threshold while also maximizing distance from DT to decrease disturbance. In this study, we experimentally evaluate DT detection by drones according to drone height, DT size, independent observers, and habitat complexity. We used 3D printed DT in three biologically realistic sizes; small (juvenile), medium (male), and large (female). We randomly selected numbers of different DT sizes into trial sets. For each experimental trial, we set 3D printed DT in a standardized sampling arena and flew the

drone from 18 to 60m above the arena, taking standardized drone images at 5m intervals. Then, the images were presented randomly to independent observers, which were asked to mark DT of various sizes from randomly drawn images using the program iTag. Unsurprisingly, we found that drone height and DT size were important factors for accurately counting DT in trials sets. We also expect that habitat complexity (i.e., open water vs. presence of surficial debris) will influence the ability of observers to accurately count DT.

Title: Patterns of Herpetofaunal Diversity in a Suburban Preserve District: Insights from a Decade of Monitoring

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Abstract: Herpetofauna are sensitive to habitat loss, fragmentation and degradation, particularly in urban ecosystems. As a result, many populations of urban herpetofauna are relegated to natures preserves embedded within the urban matrix. However, little data exists on herpetofaunal diversity in these urban nature preserves. Here we describe the results of a long-term and ongoing inventory and monitoring program from the suburbs of the third largest metropolitan region in the country: Chicago. Starting in 2009, we sampled the reptile and amphibian community at 232 permanent monitoring locations stratified across 55 nature preserves in Lake County, Illinois. From 2009—2018 we recorded 11,574 observations of 26 species via artificial cover objects, aquatic traps, and incidental encounters. Preserve richness ranged from 3 to 16 and increased with preserve size ($r^2 = 0.383$, $p < 0.001$) and habitat diversity ($r^2 = 0.385$, $p < 0.001$), but not preserve age or an index of habitat management intensity (prescribed fire, invasive species removal, etc.). The most widespread species were the American Bullfrog (n = 52 preserves) and Painted Turtle (n = 49 preserves). Five species (Common Musk Turtle, Eastern Newt, Graham's Crayfish Snake, Northern Map Turtle, and Wood Frog) were only detected at a single preserve. Our results demonstrate that urban preserves can foster high levels of herpetofaunal diversity, including rare species, such as the IUCN endangered Blanding's Turtle. However, some historically present species were notably absent (e.g. Spotted Salamander and Eastern Massasauga). We discuss best practices for long-term monitoring in urban environments and offer management recommendations.

Title: "If You Build It, They Will Come": Nest Site Enhancement as a Means of Creating More Suitable Nesting Habitat for Diamondback Terrapins in NJ

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Abstract: Anthropogenic impacts to adult female turtles have significant negative impacts for future populations. Along coastal New Jersey, diamondback terrapins (*Malaclemys terrapin*), an estuarine species, nest in upland shoreline areas. Some of these areas include roadways that bisect terrapin habitat. As a result, nesting female terrapins are prone to road mortality caused by motor vehicles. In some areas, increasing sea level rise coupled with coastal flooding can negatively impact terrapin nesting areas. In response to these threats, terrapin nest site enhancement projects have been implemented to provide safer, suitable nesting areas. The key aspect to the success of nest site enhancement is the strategic placement of nesting substrate and subsequent monitoring efforts. In some locations, such as along the Barnegat Bay shoreline – "Turtle Gardens" have been installed on both public and private properties providing alternate nesting areas for female terrapins in locations with high population densities. Many of these nest site enhancement projects in NJ have been conducted over the past five years, and their effectiveness is still being monitored. Projects along Long Beach Island, NJ have demonstrated some initial success resulting from the increase of nesting female terrapins selecting these sites, and reduction of road encounters. We feel that nest site enhancement projects can be applied to other turtle species in other areas that may have similar problems with road mortality and loss of nesting habitat.