

**Impacts of nest site choice and nest characteristics
on hatchling success in the Diamondback Terrapins
of Jamaica Bay, New York**

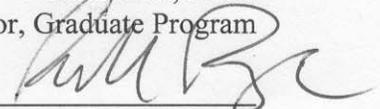
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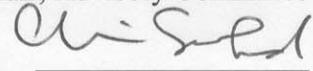
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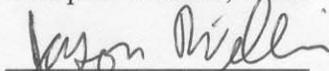
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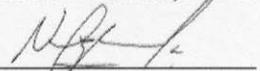
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ABSTRACT

Nest site choice and nest construction have the potential to play significant roles in the reproductive fitness of organisms. In turtles, nest site choice can affect offspring size, offspring sex, offspring locomotor performance, and offspring survivorship. I observed diamondback terrapins (*Malaclemys terrapin*) nesting at the Jamaica Bay National Wildlife Refuge in Queens, New York in 2004 and 2005. These years differed dramatically in average monthly and daily summer temperatures and rainfall. I recorded microhabitat characteristics in a one-meter area around each nest and four randomly chosen sites nearby. I also collected data on nest temperature at each nest, protected nests from predators, monitored nests for hatching, and measured hatchling survivorship.

In 2004, 1306 hatchlings emerged from 144 nests. Nest sites differed from random sites in that nest sites had significantly less overhead cover from the West. Nest temperature, measured using Pivotal Temperature Units (PTUs), was affected by the percent grass cover surrounding the nest, the nest depth and the percent dicotyledonous plant cover around the nest site. The average nest temperature during the thermosensitive period was also strongly influenced by the amount of grass cover. Emergence success (percent of eggs that resulted in hatchlings emerging from the nest) was significantly affected by the average nest temperature during the thermosensitive period, overhead cover from the East and by percent grass cover.

In 2005, 1086 hatchlings emerged from 136 nests. Nest sites differed from random sites in that nest sites had significantly less overhead cover from the South and North, significantly more bare area and significantly less leaf litter. PTUs were significantly influenced by nest depth. Average nest temperature was significantly

influenced by nest depth, overhead cover from the North, South and East and the amount of area around the nest site that was bare. Emergence success was strongly affected by nest depth.

Emergence success was not determined by female carapace size, clutch size or average egg mass for either year. Emergence success for both years was affected by nest depth, but in different ways. In 2004, warmer, shallower nests were significantly more successful and in 2005 deeper, cooler nests were significantly more successful. Nest depth was not correlated with female size, month the nest was laid, average egg weight or clutch size for either year. These differences in the impact of nest depth were due to the very dissimilar climate patterns in 2004 and 2005. Emergence success was negatively correlated with the occurrence of grass for both years, although nest depth was more important for determining emergence success in 2005 while grass cover was more important than nest depth in 2004. This study suggests that shifting climatic patterns may alter selective pressures on nesting turtles. However, grass predation may be a more consistent pressure, because grasses are important predators of terrapin eggs.

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OVERVIEW

Reptiles. Reptiles have evolved many sophisticated life history traits which help them to survive in their respective environments. The main characteristic that separates reptiles from other vertebrates is that most reptiles are ectothermic, and ectothermy plays a major role in the ecology and evolution of this group. Ectothermic animals are able to regulate their internal temperature based on the ambient temperature, and thus minimize metabolic energy use. This is opposed to endotherms, which regulate their internal heat by their own metabolic processes, and thus incur much higher energy costs (Shine, 2005).

Within class Reptilia there is wide reproductive variability, especially in squamates (lizards). Even closely related species living in the same area can have dramatically different reproductive habits. Reptiles are oviparous, ovo-viviparous, or viviparous. Oviparous reptiles lay eggs, whereas ovo-viviparous reptiles retain the egg within the reproductive tract until embryonic development is complete and then give birth to live young. A few species are truly viviparous. There is some controversy over which reproductive method evolved first. One of the earliest hypotheses is still widely accepted today; the “cold-climate hypothesis” states that vivipary evolved from ovipary in colder climates, because it is easier for ectotherms to regulate their own body temperatures and their developing embryos’ temperatures by basking than by attempting to regulate nest temperatures (Shine 2005).

Two sex determination modes are known in reptiles: environmental sex determination (ESD) and genetic sex determination (GSD). With ESD, the sex of the embryo is determined by the environment it inhabits during incubation. With GSD, the

sex of the embryo is determined by genotype at conception and is independent of environmental conditions during incubation. Temperature sex determination (TSD) is a special form of ESD where the environmental factor that is determining embryo sex is temperature. Both TSD and GSD are known in squamates and turtles, crocodilians are all TSD, and birds are all GSD (Ewert et al. 2004).

Turtles. All species of turtles are oviparous; they bury their eggs in the ground and have little or no maternal care of their young. Of 79 species of turtles that have been studied, 64 species have TSD and 15 have GSD (Ewert et al. 2004). Most TSD/GSD studies have taken place in laboratory settings where eggs were incubated at constant temperatures, which do a poor job of mimicking the process of sex determination in natural settings. Much more field work is needed to determine how fluctuating nest temperatures in the field affect hatchling sex and also how sex ratios vary on a year to year basis.

Diamondback Terrapins. The northern diamondback terrapin (*Malaclemys terrapin*) is the only completely estuarine North American turtle. Terrapins inhabit *Spartina* marshes and tidal creeks where they feed on molluscs, crabs and other crustaceans and invertebrates (Ernst et al. 1994, Brennessel 2006). Although few details are known about the aquatic ecology of *M. terrapin*, its nesting ecology has been well documented. Males mature at about 9 cm before the end of their third year and females mature at about 13.2 to 17.6cm after their sixth year (Lovich and Gibbons 1990). In New York, the nesting season begins in late May and extends to early August (Feinberg and Burke 2003). Diamondback terrapins nest primarily on isolated sandy beaches and vegetated dunes. Nesting activity increases with high tides and increased temperatures

(Burger and Montevecchi 1975; Feinberg and Burke 2003). Clutch size ranges from 4 to 18 with fewer eggs produced in the southern range and more in the northern range. Mean clutch sizes of 9.7 eggs have been recorded in New Jersey (Burger and Montevecchi 1975) and 10.9 has been recorded in New York (Feinberg and Burke 2003). Females lay 1-3 clutches per nesting season (Lazell 1979, Feinberg and Burke 2003, Scholz pers. obs.) and take around 25 minutes to do so (Feinberg and Burke 2003).

Study Site. Jamaica Bay Wildlife Refuge (JBWR) is a 3662 ha estuarine wildlife refuge located at the southwestern corner of Long Island, New York, in Jamaica Bay (Figure 1). The refuge is in the boroughs of Queens and Brooklyn, and consists of one large island, Ruler's Bar Hassock (RBH, 520 ha, 40°57' N, 73°50'W), and several smaller islands. JBWR is part of Gateway National Recreation Area (GNRA), a large, federally-operated estuarine park managed by the National Park Service. GNRA is geographically located in the Hudson-Raritan Estuary, otherwise known as the New York-New Jersey Harbor Estuary.

Although terrapin numbers are declining throughout their range (Wood and Herlands 1997), the JBWR population is still impressively large. Feinberg and Burke (2003) reported more than 2000 nests are laid there annually. Due to its size and the fact that the turtles are protected from commercial and recreational collection, JBWR is an excellent study site.

The purpose of my research is to determine why and how female diamondback terrapins choose nest sites. Also, I wanted to know how these chosen nest sites influence the temperature of the nest and the emergence success of each nest. Although incubation period and temperature have shown to affect hatchling survivorship, one of the most

important factors that affect survivorship in hatchlings is predation, both in the egg stage and as newly emerged hatchlings. At JBWR roughly 92% of nests are predated by raccoons the first night that they are laid (Feinberg and Burke 2003, Ner and Burke in press). Similarly, a study in Florida showed high rates of diamondback terrapin nest predation by raccoons (Butler et al. 2004). Therefore, this study focuses on those terrapins and nests that actually survive predation during incubation at JBWR. Similarly high levels of predation are commonly observed in turtles (Wilbur and Morin 1988).

Chapter 1

Nest Site Choice in Diamondback Terrapins

INTRODUCTION

Very little is known concerning how female turtles choose nest sites and why, but it is generally presumed that females choose nest sites so as to increase the fitness of their offspring. Some of the most important ways that female turtles can influence their embryos' nest characteristics is through their choice of nest site, when the nest is laid, and nest depth (Roosenberg 1996). Oviposition site is an important part of an animal's life history, because the area where an egg is placed may have many different impacts on incubation and the phenotype of the resulting hatchling (Madsen and Shine 1999). Research has shown that nest site choice can have an effect on hatchling incubation period (Burger 1976, Valenzuela 2001), hatchling size (Doody 1999), hatchling sex (Janzen 1994a; Kolbe and Janzen 2002; St. Juliana et al. 2004) and hatchling emergence and success (Horrocks and Scott 1991; Wilson 1998; Kolbe and Janzen 2002; Doody et al. 2004).

Some researchers have hypothesized that substrate temperature or substrate moisture content cues the female turtle to nest in a certain area (Morjan and Valenzuela 2001, Morjan 2003). For example, females nesting in warmer climates may place their nests in substrates that have a higher moisture content to protect their nests from thermal extremes. Female turtles may also use the amount of overhead cover and direct sunlight as nest site selection cues (Janzen 1994a, Kolbe and Janzen 2002). More overhead cover around a nest generally means that the nest site experiences cooler temperatures than a

nest placed under direct sunlight. Again, females nesting in warmer climates may place their nests near or under vegetation in order to protect the developing embryos from extreme heat.

Roosenberg (1996) suggested that diamondback terrapins, a species with sexual dimorphism, choose nest sites based on the size of their eggs. His rationale was that larger eggs placed in warmer areas would especially benefit the larger-bodied sex (females) and smaller eggs placed in cooler areas would not disadvantage the smaller-bodied sex, because they (males) will incur a relatively smaller cost of having small body size. This hypothesis has not been supported in subsequent analyses using diamondback terrapins (Scholz unpublished data), painted turtles (*Chrysemys picta*) (Morjan and Janzen 2003) and Amazonian giant river turtles (*Podocnemis expansa*) (Valenzuela 2001).

Reinhold (1998) hypothesized that turtle nest site choice is based on ‘nest site philopatry’, which he defined as the mechanism by which females come back to lay their eggs in the same area where they hatched. Based on this hypothesis, females would place eggs which would produce daughters in high quality nesting areas, because daughters would benefit more than sons from high quality nesting areas when they mature and return to the same beach to lay their eggs. Eggs that will produce sons would be expected in low quality habitats because sons do not benefit as much as daughters from high quality nesting habitat. Reinhold’s hypothesis was based on data from hawksbill sea turtles (*Eretmochelys imbricata*), but all sea turtles have been shown to nest on the same beaches from which they came. This hypothesis has been challenged with data from painted turtles, which is known for being a philopatric species (Valenzuela and Janzen

2001). Other research has shown that nest site affinity is a strong determinant of where female snakes will lay their eggs, regardless of the suitability of the site (Madsen and Shine 1999).

More studies are needed to determine how females choose nest sites, how the microhabitat around the nest influences the temperature of the nest and how fluctuating field temperatures affect the sex ratio, success and characteristics of the hatchlings. The purpose of this first chapter is to investigate the factors that influence female Diamondback Terrapins nest choice at Jamaica Bay, NY.

MATERIALS AND METHODS

Nesting Ecology. In June and July of 2004 and 2005 I observed female terrapins nesting on the island of Rulers Bar Hassock in Jamaica Bay (Figure 1). Females were allowed to nest naturally. Once the animal started to leave the nest site, the females were captured for processing. Processing an animal included measuring its carapace and plastron length, noting any abnormalities and giving each animal a unique PIT-tag into the coelum in front of their right hind foot, if not previously tagged. Females were also scute notched if not previously marked. The females were then released back into the water.

Nest Microhabitat Characteristics. At each nest site, on the day of oviposition, I made five measurements to determine the nest microhabitat characteristics. I visually estimated the percent bare ground, percent grasses, percent dicotyledonous plants and percent leaf litter in the 1m² square area around each nest. Because this sometimes

involved multiple layers, these percentages sometimes summed to greater than 100%. I differentiated between monocotyledonous and dicotyledonous plants because of the associated size and growth form difference between the two plant groups. I assumed that dicotyledonous plants were taller and therefore would be affecting overhead cover. Monocotyledonous plants are generally smaller and therefore I assumed that they would not have as much affect on overhead cover, but could still be assessed by nesting females as a cue to the amount of vegetation in an area. I measured also percent overhead cover directly over the nest using a spherical densiometer (see Janzen 1994a for a more complete description).

For each nest, these measurements were repeated at each of four randomly chosen locations around each nest. These random nest sites were selected by randomly choosing a direction and distance using a home-made spinner. The spinner was numbered and possible outcomes ranged from one to 12 steps (0.5-6.0 meters) in the cardinal directions.

Because female terrapins are relatively quick nesters compared to other species of turtles and leave the nest after the eggs are buried, I assumed that females were not significantly altering the nesting site. Feinberg (2000) found that the average time of nest completion, from start to finish (including digging, oviposition and covering of the nest), was 24.8 ± 6.94 min (N = 28, range = 13-47). For this reason I believe that the microhabitat variables that I measured were accurate and not the result of site disturbance by the nesting female.

Statistical Analysis. I used binary logistic stepwise multiple regression with backwards elimination to determine how terrapin nest microhabitats differed from the randomly located sites in the same habitats. Binary logistic stepwise multiple regression

was used because it allows nests and the random sites to be coded separately and compared to one another. The dependent variable was site (random vs. nest). The microhabitat variables (independent variables) entered into the model were: densiometer readings (north, south, east, west and total overhead cover (the sum of the cardinal direction readings)), percent bare ground, percent leaf litter, percent grass cover and percent dicotyledonous plant cover in the nest area. To eliminate redundant variables, a correlation matrix was created for all independent variables, also eliminating the problems of multicollinearity. One variable among a highly correlated pair ($r > 0.80$) was retained in the regression model. I used SPSS[®] for Windows version 12.0 for all statistical analyses.

RESULTS

2004. In 2004 I observed 143 terrapin nestings. Terrapin nests differed significantly from randomly chosen sites only in that nest sites had significantly less overhead cover to the West ($P < 0.001$) (Table 1). Total densiometer reading (sum of North, South, East and West densiometer readings) was removed from the overall model because of its significant correlations with other independent variables ($r > 0.8$) and because it had the least predictability when kept in the model. The main model also showed other correlations between variables, but these correlation values were not high enough ($r > 0.8$) to warrant their removal from the overall model.

However, for this reason these variables were inspected pair-wise using Pearson correlation analysis to determine whether these variables were significantly correlated with each other. The percent bare area and the total densiometer readings were

significantly negatively correlated ($r(571) = -0.647, P < 0.001$). In general, the more bare ground in an area, the less overhead cover there. The percent bare area and the percent dicotyledonous plants in an area were also significantly negatively correlated ($r(571) = -0.724, P < 0.001$). The more bare in an area, the fewer dicotyledonous plants there. The total densiometer readings were also significantly correlated with the amount of dicotyledonous plants in the area ($r(571) = 0.780, P < 0.001$). The more dicotyledonous plants were associated with more overhead cover there. Other variables were significantly correlated, but the correlations were weak ($r < 0.4$).

2005. In 2005 I observed 138 terrapin nestings. Terrapin nest sites differed significantly from randomly chosen sites by four variables: less overhead cover to the South ($P < 0.001$) and North ($P = 0.055$), more percent bare area around the nest ($P = 0.028$) and less percent litter around the nest ($P < 0.001$) (Table 2). Again, total densiometer readings were removed from the model because of significant correlations with other microhabitat variables ($r > 0.8$). The main model revealed correlations between some of the variables, but these were not high enough ($r > 0.8$) to warrant being removed from the model. The percent bare area and the total densiometer readings were significantly negatively correlated ($r(690) = -0.716, P < 0.001$). In general, the more bare ground in an area, the less overhead cover in that area. The percent bare area and the percent dicotyledonous plants in an area were also significantly negatively correlated ($r(690) = -0.782, P < 0.001$). The more bare an area, the fewer dicotyledonous plants in the area. The total densiometer readings were also significantly correlated with the amount of dicotyledonous plants in the area ($r(690) = 0.851, P < 0.001$). Areas with

more dicotyledonous plants had more overhead cover. Other variables were significantly correlated, but the correlations were weak ($r < 0.3$).

DISCUSSION

My results are both corroborative and different than the results of past papers on the subject. Janzen (1994a) studied painted turtles (*Chrysemys picta*) at approximately the same latitude. He found that one hatchling characteristic (sex ratio) was mostly influenced by overhead cover (also measured using a spherical densiometer) from the West and South, and therefore inferred that nesting females used overhead cover cues when choosing nest sites. At Janzen's site, beaches with more overhead cover produced mostly male-biased nests compared to beaches with less overhead cover that produced mostly female-biased nests. Janzen (1994a) did not look at how nest sites differed from random at his study site and I did not measure hatchling sex ratio, so these parts of the analysis are not comparable. St. Julianna et al. (2004) found that snapping turtle (*Chelydra serpentina*) hatchling sex ratio was most influenced by the solar exposure from the South, East and the total solar exposure. St. Julianna et al.'s study, which took place near Jansen's (1994a) site, also did not look at how nest sites differed from non-nest sites and did not focus on female nest site choice.

Kolbe and Janzen (2002) also studied nest site choice in snapping turtles in the same county as the previous two papers. They found that in natural, non-disturbed sites, females chose nest sites non-randomly (using a spatial distribution model) and nest temperatures and hatchling sex ratios were correlated with the nest microhabitat

characteristics that were measured. Nest sites were characterized by shorter vegetation height, more bare ground, less litter, less herbaceous vegetation, less succulent vegetation, less dry vegetation mass and fewer cacti compared to random locations. Nest sites also differed in having less total overhead cover than random nest sites.

I found that in 2004, the main differences in terrapin nest sites and between randomly generated sites was that there was more solar exposure from the West. No ground-based microhabitat variables were associated with nest site choice in 2004. But I found that in 2005, the difference between nest sites and random sites was based on multiple factors, including less solar exposure from the North and South, (but not from the West as found in 2004 and in the Janzen's (1994a) site nor from the East as found in St. Julianna's site (2004)). Again, these studies were looking at hatchling sex ratios and not nest site choice, which was my focus. However, it may be possible that the specific compass direction of canopy openings is not particularly important, and the important conclusion from these results is that the turtles look for gaps in the overhead canopy cover when choosing nest sites.

I also found that the amount of bare ground and leaf litter cover differed significantly around terrapin nests compared to random sites in 2005. Females nested in areas with more bare ground and less litter. Janzen (1994a) did not record many of the other microhabitat characteristics (percent bare ground, percent leaf litter, percent grass cover and percent dicotyledonous plant cover) that I measured, so I cannot compare my results directly to his. Others studies have measured these variables (St. Juliana et al. 2004), but nest site selection was not examined in these studies. My results from 2005 concur with Kolbe and Janzen (2002), who found that nest sites differed from random

sites by having more bare ground and less litter. Wilson (1998) found the opposite results, where female striped mud turtles (*Kinosternon baurii*) were more likely to choose nest sites closer than random to grass tussocks and herbaceous vegetation. She suggested that this was due to the small body size of the turtle, which resulted in the turtle only being able to build shallow nests, and depended upon nest vegetation to cool and protect the nest from thermal extremes. Other studies of diamondback terrapins in the Northeast have also shown that females tend to lay their nests in sunny areas away from vegetation (Burger and Montevecchi 1975; Roosenberg 1996).

I chose to measure these microhabitat specific variables because they have been shown in previous studies to influence the nest environment. Vegetation around the nest site has been previously shown to have an effect on the temperature of the nest: the more vegetation surrounding a nest, the lower the nest temperature is likely to be (Kolbe and Janzen 2002, St. Juliana et al. 2004). Soil moisture is also negatively correlated with nest temperature and has been shown to affect nest temperatures (Morjan 2003).

Vegetation can have a detrimental effect on hatchling emergence through egg predation (Lazell and Auger 1981; Stegmann 1988; Bouchard and Bjorndal 2000). Diamondback terrapin nests are often predated by beach grass, *Ammophila breviligulata* (Lazell and Auger 1981, Stegmann 1988, Feinberg and Burke 2003). Conversely, a study of sea turtle nest success showed that increased vegetation at the nest site allowed for greater hatchling emergence because the sand was less compacted (Horrocks and Scott 1991). More bare ground around the nest indicates less vegetation, and conversely, more dicotyledonous plants indicate more overhead cover. Although correlation analysis showed no extremely strong correlations ($r > 0.8$) between any of the microhabitat

variables and overhead cover, these variables were still significantly correlated with one another, in that reduced overhead cover corresponded with less ground plant growth and may also influence the amount of leaf litter and bare ground.

Female turtles of many species, including female terrapins, have been documented “throwing sand” with their fore limbs and “ground-nuzzling” before choosing nest sites (Morjan and Valenzuela 2001, Scholz pers. obs.). Female terrapins at our study site have been seen exhibiting this behavior for over an hour before finally depositing their nests (Scholz pers. obs.). When turtles perform this behavior they may be evaluating the substrate and in effect determining an optimal place to lay their nest. It has been demonstrated that when turtles perform this behavior, they are not determining the surface soil temperature (Morjan and Valenzuela 2001). However, the amount of bare ground and litter around a nest might be easy to estimate using this “ground-nuzzling” method. Both variables are good indicators of the amount of vegetation in the area.

It appears that nesting turtles may be using overhead cover cues in combination with microhabitat variables to help determine where to place their nests. Although the visual capabilities of turtles is mostly unknown, I, along with Janzen (1994a), St. Juliana et al. (2004) and Kolbe and Janzen (2002), found that measures of overhead cover are important to the female when choosing nest sites, and solar exposure in general may be one environmental characteristic that turtles use to determine nest sites. It may be that females aren't using any specific direction of solar exposure, but just determining the general overhead cover based on the time during the day that they are laying their nests.

The fact that females appear to be using different cues in different years is interesting. In both years females are using overhead cover as a useful cue for nest placement. For this reason I believe that this is commonly used cue and will be used by females in the future. In 2005 females were also using vegetational cues for nest placement. The difference could be due to the fact that the environment is ever-changing at our study site. Each year more invasive vegetation takes over and more dune area (suitable nesting area) erodes away (Scholz pers. obs.). For this reason females may need to use different cues in different years to find the optimum nest site.

Based on my 2005 results and Kolbe and Janzen (2002), it also appears that females are using terrestrial microhabitat cues to determine where to lay their nests and they are generally placing their nests in areas with less vegetation and more bare ground. Although nocturnal terrapin nesting has not been observed at JBWR (Feinberg and Burke 2003, Burke pers. obs., Scholz pers. obs.), terrapins are known to nest at night elsewhere (Draud pers. obs., Auger and Giovannone 1979; Wood and Herlands 1997). It is not clear exactly how turtles evaluate potential nesting areas. Most of the recent work on turtle vision has centered on neuro-stimulation of the eye, usually in red-eared sliders (*Pseudemys scripta elegans*), which are closely related to terrapins. These studies have shown that turtles can see color and, in the case of red-eared sliders, are more sensitive to the blue end of the spectrum, which is used to see on land (Granda et al. 1986; Ammermuller et al. 1995). Also, other research on red-eared sliders has shown that they are as sensitive to UV light as are other vertebrates (Ammermuller et al. 1998) and can see at night as well as humans (Granda et al. 1995). More research is needed on turtle

visual capabilities in order to determine exactly how females perceive the nesting environment and how they use that vision to determine where to place their nests.

Chapter 2

The Effect of Nest Site Choice on Nest Temperatures

INTRODUCTION

Incubation temperature can have extremely important effects on turtle hatchlings. These effects include impacts on body size (Rhen and Lang 1999), locomotor ability (Doody 1999; Janzen 1993; Ashmore and Janzen 2003), hatching success rate (Wilson 1998; Cagel et al. 1993) and yolk reserves (Rhen and Lang 1999). Many species of turtles, including diamondback terrapins (*Malaclemys terrapin*), have temperature-dependent sex determination (TSD) (Valenzuela 2004a), so incubation temperature can also impact sex. In animals with TSD, sex is determined after fertilization by the incubation temperature that the embryo experiences during a short window of time called the thermosensitive period (Mrosovsky and Pieau 1991). There are two types of TSD found in turtles. In TSD I females are produced at warm temperatures and males are produced at cooler temperatures; TSD II is the exact opposite (Ewert et al. 2004). Diamondback terrapins exhibit TSD I (Roosenberg and Kelly 1996), with 28°C being the pivotal temperature (Giambanco 2004).

Research on TSD has increased dramatically in recent years. Survey work on 79 species of turtles has shown that 64 have TSD (Ewert et al. 2004). Nearly all TSD research has been carried out in laboratory settings, where it is relatively easy to determine the effect of temperature on hatchlings incubated at constant temperatures. These studies have determined how controlled temperatures affect incubator-produced hatchlings. This is how TSD, in both its forms, was discovered in many turtle species.

Researchers have tested the effects of incubation temperature on nests in the field in order to understand the biological relevance of this phenomenon (e.g., painted turtle *Chrysemys picta* [Kolbe and Janzen 2002; Morjan 2003], striped mud turtle *Kinosternon baurii* [Wilson 1998], giant river turtle *Podocnemis expansa* [Valenzuela 2001], pig-nosed turtle *Carettochelys insculpta* [Doody et al. 2004]). In the field the temperatures of the nests are not constant and can vary in complex ways. It is possible that characteristics such as hatchling size and sex may have much more complex relationships with incubation temperature under the varying temperature regimes commonly experienced in the field.

Female turtles can have a direct impact on nest conditions such as nest temperature and predation risk through nest-site selection. Nest site selection for turtles has been studied in only a few species (*Caretta caretta*: Wood and Bjorndal 2000; *Caretta insculpta*: Doody et al. 2003; *Chelydra serpentina*: Kolbe and Janzen 2002; *Chrysemys picta*: Janzen 1994a, Morjan 2003; *Kinosternon baurii*: Wilson 1998; *Malaclemys terrapin*: Burger and Monevecchi 1975, Roosenberg 1996; *Podocnemis expansa*: Valenzuela 2001). From these studies it is evident that site selection can greatly affect the sex ratio and success of the hatchlings, presumably through the influence of incubation temperature.

Temperature data from field nests are complicated and show strong variation in daily and seasonal patterns, short- and long-term weather events, and plant growth patterns, all of which change over the entire incubation period. Studies of the effect of nest temperature on offspring sex have focused on the thermosensitive period (TSP), which simplifies analysis of field temperature patterns somewhat and emphasizes

measurements during a critical period of hatchling development. Schwartzkopf and Brooks (1985) showed that measures as simple as average nest temperature are unable to correctly predict offspring sex. Studies that used mean July nest temperatures did so because July happened to be the TSP period for the turtle species being studied. For example, Kolbe and Janzen (2002) used mean July nest temperatures to successfully compare nest temperatures to hatchling sex ratios in painted turtles. Morjan (2003) used minimum, maximum and mean July nest temperatures when looking at nest site choice in painted turtles. Cagle et al. (1993) also used minimum, maximum and mean nest temperatures to determine that the availability of water in nests is more important than temperature in influencing survival, metabolism and growth in painted turtles. Mullins and Janzen (2006) showed that incubating hatchlings at different fluctuating temperatures can have widespread effects on their phenotypes. Few field studies have used much more than maximum, minimum and average nest temperatures to determine how temperatures differ between sites and how incubation temperatures affect hatchlings. Therefore, there is no standard measure of field nest temperatures that has been shown to reliably predict the type of effects (e.g., hatchling body size, locomotor ability, hatching success rate, yolk reserves) known to result from variation in laboratory-incubated eggs.

More recently, Valenzuela (2001) developed a method for relating incubation temperatures to hatchling sex. This was done by taking into account all of the hours that the embryos experience above the species threshold temperature, which is the temperature at which below no embryos survive. These Critical Thermal Units (CTUs) are found by taking the number and magnitude that a temperature reading was above the threshold temperature and then summing the units. CTUs have been used successfully

for determining the sex ratio of nests of an Amazonian river turtle, *Podocnemis expansa* (Valenzuela, 2001). A variation of this model, Constant-Temperature Equivalents (CTEs) has been used to relate incubation temperature profiles to hatchling sex (Georges et al. 2004). CTEs summarize the impact of nest temperature as a function of both the number of hours during the incubation period that the nest was above the pivotal temperature (the temperature at which approximately equal numbers of male and female offspring are produced) and the number of degrees each hourly temperature reading is above the pivotal temperature. CTEs have been used successfully for determining the sex ratio of nests of the pig-nosed turtle from Australia, *Carettochelys insculpta* (Doody et al. 2004).

Therefore, calculating an index of incubation temperature based on the degree hours that the temperature is above some critical point appears to be a good way to measure the impact of incubation temperature on a key hatchling characteristic, hatchling sex. However, neither CTEs nor CTUs have been previously used to measure the impact of incubation temperature on other hatchling characteristics (i.e. phenotype, growth rate, hatchling survivorship). Because the survivorship threshold temperature for diamondback terrapins is unknown, I used a modified version of CTUs. Instead of measuring the amount of time that temperatures were above the threshold temperature, I measured the amount of time that temperatures were above the pivotal temperature and deemed these Pivotal Temperature Units (PTUs). The purpose of this chapter was to study how nest site selection affected the temperature, measured both in PTUs and average temperature during the TSP, of Diamondback Terrapin nests.

METHODS

Microhabitat variables and Nest Excavation. I observed diamondback terrapins nesting at Jamaica Bay Wildlife Refuge; at each nest site I made eight measurements of the nest microhabitat in a one-meter circular area around each nest: percent bare ground, percent grasses, percent dicotyledonous plants, percent leaf litter, and overhead cover to the North, East, South, West and total, as described in Chapter 1. Then I excavated all nests on the day they were oviposited and counted and weighed the eggs. Because the eggs were moved on the day of oviposition I assumed the movement would have no detrimental effect on the embryos (Ewert 1979). I measured nest depth and then replaced the eggs in the nest in their original oviposition positions and then placed an ibutton® temperature logger on top of the eggs before re-burying them with the original soil to their original depth. The temperature loggers recorded the temperature of the nest every hour with 0.5°C accuracy. I recovered the temperature loggers once the hatchlings emerged.

Statistical Analysis. The temperature loggers I used were unable to record hourly temperatures for the entire incubation period of some nests; therefore I focused on the temperatures during the middle third of incubation as a proxy for the entire incubation period. The middle third of incubation has been shown to be the Temperature Sensitive Period (TSP) during which sex is determined in other turtle species (Bull 1987, Mrosovsky & Pieau 1991) and thus is likely to be important to other hatchling traits. I estimated the TSP for each nest by assuming the incubation period was the number of days from oviposition until the day of the first hatchling emergence, and then divided the

incubation period into thirds, and assumed that the TSP was the middle third of the incubation period of each nest individually.

I calculated Pivotal Temperature Units (PTUs) during the TSP as a function of both the amount of time that each temperature record was above the pivotal temperature (28°C) and how far above each record exceeded 28°C. I subtracted 28 from each temperature record that exceeded this value, and added the resulting values (modified from Valenzuela 2001). I calculated the average temperature for each nest by first determining the thermosensitive period (TSP). Then, the hourly temperature readings from the TSP were averaged.

I used stepwise multiple regression with backwards elimination to determine which of the nine nest microhabitat variables and nest depth, affected average nest temperature (dependent variable). The microhabitat variables (independent variables) entered into the model were: percent bare ground, percent grasses, percent dicotyledonous plants, percent leaf litter, and overhead cover to the North, East, South, West and total. Nest depth was also added to the model as an independent variable. To eliminate redundant variables, a correlation matrix was created for all independent variables. Only one variable among any highly correlated pair ($r > 0.80$) was retained in the regression model. The same process was used to determine how the microhabitat variables and nest depth affected the nest PTUs during the thermosensitive period (dependent variable). All statistical analyses were done with SPSS[®] for Windows version 12.0.

I examined temperature, precipitation and cooling degree days records for June, July and August from 1984-2005 from the JFK International Airport weather station. I

ranked the records in increasing magnitude and compared records from 2004 and 2005 to the previous 20 years.

RESULTS

2004: I collected temperature data from 73 nests. Average female carapace length was 20.2 ± 1.5 cm (N = 140, minimum = 18cm, maximum = 25cm) (s.d.). Average nest depth was 15.2 ± 1.9 cm (N = 138, minimum = 9cm, maximum = 20cm) (Figure 1). Average pivotal temperature units (PTUs) were 432.8 ± 230.5 (N = 73, minimum = 8.5, maximum = 1082) (Figure 3). The PTUs (Figure 2) in nests were significantly influenced by the percent area around the nest with grass (P = 0.008, $r^2 = 0.080$), dicotyledonous plants (P = 0.047, $r^2 = 0.016$) and nest depth (P = 0.055, $r^2 = 0.028$) (Figure 12) (Table 4). Average temperature during the TSP was 25.83 ± 1.0 (N = 73, minimum = 23.3, maximum = 27.5) (Figure 4). Average temperatures during the TSP (Figure 3) were also significantly influenced by the percent grass around the nest (P = 0.039, $r^2 = 0.060$) (Table 3). Figure 13 shows the relationship between nest depth and average nest temperature during the TSP in 2004. The total densiometer variable was excluded from the regression model because it was significantly correlated with other readings ($r > 0.8$) (see chapter one for the justification behind the elimination).

2005: I collected temperature data from 122 nests. Average female carapace length was 19.6 ± 0.9 cm (N = 137, minimum = 17.8cm, maximum = 22cm).

Average nest depth was 14.9 ± 2.2 cm (N = 138, minimum = 9.5cm, maximum = 20cm) (Figure 4). Average PTUs were 785.1 ± 371 (N = 122, minimum = 83.5, maximum = 2224.5) (Figure 6). PTUs (Figure 5) were significantly influenced by the nest depth (P < 0.001, $r^2 = 0.239$) (Table 6) (Figure 14). Average temperature during the

TSP was 28.2 ± 1.3 (N = 122, minimum = 25.0, maximum = 30.7) (Figure 7). Average temperature during the TSP (Figure 6) was significantly influenced by the nest depth (P = 0.001, $r^2 = 0.082$) (Figure 15) overhead cover from the North (P = 0.013, $r^2 = 0.054$), South (P = 0.085, $r^2 = 0.003$) and East (P = 0.043, $r^2 = 0.008$) and also by the percent area around the nest that was bare (P = 0.095, $r^2 = 0.008$) (Table 5).

In comparison to the climate records from JFK Airport weather station from 1984 to 2005, 2004 was fairly wet (in the upper 10th percentile in total precipitation) and cool (lower 25th percentile for average temperature and lower 20th percentile in cooling degree days). In contrast, 2005 was fairly dry (lower 15th percentile in total precipitation) and hot (upper 90th percentile in average temperature and upper 90th percentile in cooling degree days).

DISCUSSION

Some variables affected PTUs and average nest temperature during the TSP consistently and some did not. In 2004 PTUs were inversely affected by the percent of area around the nest that contained grass, dicotyledonous plants and nest depth. In 2005, PTUs were strongly inversely affected by the depth of the nests. Both percent grass and nest depth were inversely correlated with PTUs in both years.

In 2004, average nest temperatures during the TSP were significantly and positively affected by the percent grass around the nest: the more grass around a nest, the lower its average temperature. But, in 2005, average nest temperature during the TSP was primarily affected by nest depth (inversely) and overhead cover from the North (inversely), South (inversely) and East (inversely) and also the percent bare area around the nest (positively). These results are consistent with chapter one in that females may be

using both the amount of overhead cover and vegetation cues to determine where to place their nests. But, nest temperature was not measured in random locations and therefore I cannot say that females were choosing locations based on some optimum temperature.

Janzen (1994a) similarly measured overhead cover and also hatchling sex ratios in painted turtles (*Chrysemys picta*). He did not record nest temperatures. He assumed that because he found more male-biased sex ratios in nests with more overhead cover, overhead cover influenced the sex ratio of the nests and may have been the cue that female turtles used in nest site selection. St. Juliana et al. (2004), when studying snapping turtles (*Chelydra serpentina*), recorded many of the same microhabitat variables as in this study, and found that temperatures were correlated most strongly with densiometer readings from the South, East and total densiometer readings. Kolbe and Janzen (2002) also carried out a study much like mine, and found that mean nest temperatures during the TSP were significantly correlated with the percentage of overhead vegetation cover at one site and then at another site the temperature was significantly correlated with the amount of vegetation surrounding the nest

These studies and mine (Chapter 1) indicate that females may use solar exposure and vegetation cues for nest placement. Solar exposure is a good indicator of the amount of vegetation surrounding a nest site; as shown in chapter one, the more vegetation in an area, the higher the densiometer readings. Also, the amount of bare ground in an area is negatively correlated with overhead cover (densiometer readings). The presence of grass around the nest and overhead cover from the North were important indicators of future nest temperature at JBWR and I infer that females were choosing nest sites on the basis

of the absence of plant cover. They could accomplish this by assessing overhead cover or by observing plants, leaf litter, debris, and bare sand at ground level.

I found that microhabitat variables had many different relationships with PTUs and average TSP temperature in 2004 and 2005; these differences could have been due to the climatic differences between the two summers. Compared to the previous 20 years, the summer of 2005 was abnormally warm and 2004 was abnormally cool. In 2004 females chose nest sites with significantly less overhead cover than random sites (Chapter 1), but nest temperatures were influenced by the vegetation in the nest area, not overhead cover. In 2005, females again chose nest sites with significantly less overhead cover, and also more bare ground and less litter than random sites (Chapter 1), but nest temperatures were significantly influenced by the nest depth, presence of bare ground and overhead cover. Thus there was no entirely consistent pattern of female nest site choice.

Based on the results of the first chapter, it is apparent that nesting females probably use various cues to place their nests in relatively open areas with relatively little vegetation, thus seeking warm areas to lay their nests. These results indicate that this is a generally successful strategy for selecting nest sites that will be warm through at least the middle third of incubation. Since little is known concerning the visual capabilities of turtles, it is hard to say whether females are able to assess difficult or distant cues, such as layers of terrestrial vegetation or overhead cover, especially at night. Recent studies have shown that turtles can see color (Granda et al. 1986; Ammermuller et al. 1995), are as sensitive to UV light as are other vertebrates (Ammermuller et al. 1998) and can see at night as well as humans (Granda et al. 1995). So while nesting females appear to be using visual cues to determine where to place their nests, it is still unknown exactly how

well they are able to see and distinguish between microhabitat variables when deciding where to place their nests. More research is needed on turtle visual capabilities in order to determine exactly how females perceive the nesting environment and how they use that vision to determine where to place their nests. But from previous research on painted turtles, it appears that these are cues that females could be using even at night to determine where to place their nests. From my research it appears that females are using both vegetation and overhead cover as cues, which will have a strong influence on local nest temperatures.

Despite the strong relationships between nest site conditions at oviposition and later nest incubation conditions, it is interesting to note that there is a considerable level of variability in nest conditions. For example, two nests in 2005, nests #103 and #105, had very similar densiometer readings (all zeros, indicating no overhead cover) and terrestrial microhabitat variable levels (85% bare ground, 5% litter and 10% dicotyledonous plants), but nevertheless had very different PTUs (1180.5 and 638, respectively) and average TSP temperatures (30.54°C and 28.63°C , respectively). Both nests were also laid on the same day, within 5 minutes of one another and were located in the same general area. The main difference in the nests was the nest depth, nest #103 was 15.5 cm deep and nest #105 was 17.5cm deep. Nest #103 had 12 eggs and nest #105 had 9 eggs. The female that laid nest #103 had a carapace length of 20.4cm and the female that laid nest #105 had a carapace length of 18.4cm. So, the larger turtle laid more eggs, but dug a shallower nest and therefore those eggs experienced higher temperatures. The smaller turtle dug a deeper nest and deposited fewer eggs and those eggs experienced lower temperatures. This example demonstrates that even when

females nest in the same general area, they can still manipulate the temperatures of their nests by altering nest depth. But, females have also been seen digging shallower nests on extremely warm days (Scholz pers. obs.), so there may be other factors which were not measured that could be having an effect on how deep a female nests.

Even if cues used by nesting females had not varied extensively between years, incubation conditions can vary tremendously due to variable annual weather patterns. This result will become even more important as climatic patterns change dramatically and rapidly, as predicted for this region. Based on current trends, if the warming trend from the last century continues during this century, the average annual temperature will increase by 1.0⁰F (0.56⁰C) by the 2020s, 1.5⁰F (0.833⁰C) by the 2050s and as much as 2.5⁰F (1.389⁰C) by the 2080s (Rozenweig and Solecki 2001). Research on painted turtles has shown that an increase of 2⁰C will significantly skew sex ratios and an increase more than 4⁰C will stop the production of male offspring in species with type Ia TSD (Janzen 1995b), such as diamondback terrapins. Rapid behavioral and evolutionary adaptation will be needed to accommodate to these changes.

Chapter 3

How Nest Site Choice affects Emergence success

INTRODUCTION

Numerous turtle hatchling characteristics are affected by incubation temperatures. Incubation time is negatively correlated with average nest temperature, such that under otherwise constant temperature conditions, warmer nests have shorter incubation durations than colder nests (Valenzuela et al. 1997; Mullins and Janzen 2006). Hatchling size is also negatively correlated with incubation temperature, such that warmer temperatures tend to result in smaller hatchlings (Cagle et al. 1993; Rhen and Lang 1999; Doody 1999; Mullins and Janzen 2006; Ji et al. 2003). These characteristics are important because reducing incubation duration may reduce predation risk; and hatchling size is related to locomotor performance (Janzen et al. 2000; Doody 1999; Freedberg et al. 2004; Mullins and Janzen 2006) and perhaps also survivorship (Ashmore and Janzen 2003). Hatchling survivorship in the nest is also dependent upon temperature, where hatchlings cease to hatch at extremely cold and extremely warm temperatures (Ji et al. 2003; Mullins and Janzen 2006). The nesting environment in the field (i.e. temperature and vegetation) can also have an affect on turtle hatching success (Wilson 1998; Kolbe and Janzen 2001; 2002; Burger 1977; Horrocks and Scott 1991; Cagle et al. 1993; Doody et al. 2004). Hatchling growth rate can also be affected by the nesting environment (Brooks et al. 1991). Incubation temperature can also affect subsequent reproductive behavior (Gutzke and Crews 1988 [Leopard Geckos, *E. macularius*), thermal preferences (Rhen and Lang 1999), anti-predator behavior (Doody

1999), and hatchling sex (Valenzuela 2004). All of these characteristics can have important impacts on survivorship and reproduction. Several of the relationships described above have only been demonstrated in laboratory settings, and none have been well studied in field conditions. One of the most important differences between field conditions and laboratory experiments is the difference between incubation temperatures used in laboratory experiments and irregularly fluctuating incubation temperatures experienced in the field. There is a strong need for more studies to determine how hatchlings are affected by constantly fluctuating field temperatures (Valenzuela 2004).

In this study I sought to determine how emergence success, a critical aspect of reproductive fitness, was affected by nest temperatures and microhabitat variables of natural diamondback terrapin nests.

MATERIALS AND METHODS

Nest Protection. I observed terrapins nesting at Jamaica Bay Wildlife Refuge, measured microhabitat variables around each nest, and excavated nests (chapters 1 and 2). I measured female plastron and carapace lengths and implanted PIT tags inter-coelomically, if necessary, before releasing terrapins back into the water. I counted and weighed the eggs, measured nest depth, replaced the eggs in the nest in their original oviposition positions and then placed an ibutton® temperature logger on top of the eggs. I re-filled the nests with the original soil, and protected them from egg predators with predator excluders. In 2004, I protected nests using a square flat piece of 1.27cm hardware cloth measuring 61 by 61 cm held to the ground with metal stakes. Sixty days post-oviposition, I removed the hardware cloth and placed a 40.6 by 40.6 cm predator

excluder, 5.08 cm high, on top of the nest (Figure 10). I also staked this predator excluder into the ground using metal stakes. In 2005 I protected nests with a circular cage made with 1.27 cm hardware cloth measuring 60 cm deep with a diameter of 45 cm. I placed these predator excluders 10 cm into the ground around the nest and staked them into the ground (Figure 11). These nest exclosures were assumed to have no effect on nest temperature, but this was not actually investigated in this study.

Hatchlings. I monitored nests daily until hatchling emergence. If no hatchlings emerged, I excavated each nest when it was 105 days old, except eight nests in 2004, which did not have any temperature loggers in them and were left to over-winter. Nests were also excavated after all the hatchlings had emerged or ten days after the last hatchling had emerged. I counted all unhatched eggs and hatchlings (live or dead) when I excavated the nests, and I recorded any root predation.

Statistical Analysis. I defined emergence success as the percent of oviposited eggs that resulted in hatchlings that emerged from the nest. I calculated PTUs and average nest temperature during the thermosensitive period as described in Chapter 2. I used stepwise multiple regression with backwards elimination to determine which variables had significant effects on emergence success (dependent variable). To eliminate redundant variables, a correlation matrix was created for all independent variables. One variable among a highly correlated pair ($r > 0.80$) was retained in the regression model. The independent microhabitat variables I entered into the model were: densiometer readings (north, south, east, west and total), percentage of the area around the nest that was bare, percentage of the area around the nest that was covered with leaf litter, percentage of the area around the nest that was covered with grass and percentage

of the area around the nest that was covered with dicotyledonous plants. I also added nest depth, PTUs and average temperature during the TSP for each nest to the model as additional independent variables.

I used simple linear regressions to determine whether the vegetation microhabitat variables affected the likelihood of root predation. I also used simple linear regression to investigate whether female body size, egg size or clutch size significantly influenced nest depth. I also used a separate independent sample t-test to determine whether the month the nest was laid influenced nest depth.

I also used chi-square tests to see the difference in egg desiccation and hatchling mortality inside the nest for both years and Spearman Correlation Coefficient to determine how microhabitat variables were correlated. Independent-sample t-tests were also used to determine if there was significant differences between nest depth and incubation periods for the two years. For those females for which I had data on multiple nests, I use a repeated measures model to determine how their nesting characteristics differed between the two years and within the same year. I ran all statistical tests in SPSS[®] for Windows version 12.0.

RESULTS

2004. I protected 144 terrapin nests containing a total of 1856 eggs. Of these nests, 73 had temperature loggers placed in them that had resulted in successful temperature readings. Of these eggs, 1306 (70.4%) emerged as hatchlings from their nests. Of the eggs that failed to hatch, 77 (4%) were infertile/unhatched eggs, 97 (5%) were root predated, 97 (5%) eggs were flooded, and raccoons, despite nest protection,

predated 263 eggs. Root-predated eggs were found in 23 nests, with one to 15 eggs predated in each nest. Raccoon-predated hatchlings, those that were predated even through the nest excluders, were found in 21 nests ranging from one to 16 hatchlings predated per nest. Some hatchlings were found alive in the nest upon excavation (N = 156) and 32 hatchlings were found dead in the nest upon excavation. I could not account for 95 eggs that I initially counted and measured; these were distributed evenly among the protected nests and there were no more than two eggs missing in any one nest. I conclude these missing eggs had hatched and the hatchlings escaped because there were no unhatched eggs left in the nest and although the reconstruction of egg shells was attempted, there still appeared to be missing hatchlings. Therefore, I used 1187 hatchlings for the emergence success analysis. Table 7 shows the fate of all eggs in both years.

Nests took an average of 76 ± 10 days (N = 144, range = 63 to 105 days) (s.d.) from oviposition to first emergence, not including those I allowed to overwinter. The average nest depth was 15.2 ± 1.9 cm (N = 143, range = 9 to 20cm).

Of the eight nests that I allowed to overwinter, some hatchlings emerged in the fall from only four nests; no hatchlings subsequently emerged in the spring from these nests. Hatchlings emerged in the spring from two of the nests with no fall hatchling emergence. In one of these nests, only one hatchling emerged out of a nest of 11 eggs, in the other nest, 10 of 11 eggs emerged. No hatchlings emerged from the final two nests.

The backward stepwise multiple regression model evaluating the impact of all four microhabitat variables (percentage of the area around the nest that was bare, percentage of the area around the nest that was covered with leaf litter, percentage of the

area around the nest that was covered with grass, and percentage of the area around the nest that was covered with dicotyledonous plants), all five densiometer readings (north, south, east, west and total), nest depth and nest temperature (measured in PTUs and average nest temperature during the TSP) on emergence success rate, showed that the percent grass around the nest ($p = 0.038$), densiometer readings from the East ($p = 0.056$) and average nest temperature during the TSP ($p = 0.032$) had the most significant affect on hatchling survivorship (cumulative $r^2 = 0.212$) (Table 8). The total densiometer reading was left out of the model due to their high correlation with other densiometer readings and microhabitat variables. The model also revealed that emergence success was significantly correlated with PTUs ($r(73) = 0.304$, $p = 0.009$) and nest depth ($r(73) = -0.185$, $p = 0.030$).

The incidence of root predation was significantly affected by the percent area around the nest site that contained grass ($r^2 = 0.066$, $p = 0.002$). As the amount of grass around the nest increased, so did the amount of root predation that the eggs in the nest experienced. Root predation was not significantly affected by the amount of bare area surrounding the nest ($p = 0.101$, $r^2 = 0.019$), although the percent bare ground was significantly negatively correlated with the presence of grass ($r(73) = -0.514$, $p < 0.001$) (Figure 7). Root predation was also not significantly affected by the nest depth ($r^2 = 0.005$, $p = 0.429$).

Nest depth was not dependent upon female carapace length ($r^2 = 0.000$, $p = 0.861$), the month in which the nest was laid ($F = 0.211$, $p = 0.647$), egg size ($r^2 = 0.001$, $p = 0.777$) or clutch size ($r^2 = 0.009$, $p = 0.270$).

Clutch size was not dependent upon female carapace size ($N = 140$, $r^2 = 0.001$, $p = 0.754$). Average egg size was dependent upon female carapace size ($N = 140$, $r^2 = 0.267$, $p = 0.000$). Emergence success was also not dependent upon female carapace size ($N = 138$, $r^2 = 0.010$, $p = 0.236$). Emergence success was also not determined by clutch size ($N = 141$, $p = 0.132$, $r^2 = 0.016$) or average egg mass ($N = 141$, $p = 0.174$, $r^2 = 0.013$).

In 2004 there were five females for which I had data on two clutches for the summer. However, I was unable to determine which were first, second or third clutches. Emergence success was not influenced by whether the clutch was early or late ($F = 0.014$, $df = 4$, $p = 0.911$). The depth of early nests was significantly deeper than later nests ($F = 18.655$, $df = 3$, $p = 0.023$). Neither clutch size (number of eggs) nor clutch mass differed between the clutches ($F = 0.419$, $df = 4$, $p = 0.553$ and $F = 0.001$, $df = 4$, $p = 0.977$, respectively).

2005. I protected 136 nests containing 1724 eggs in 2005. Of these nests, 122 had temperature loggers with successful temperature readings. 1087 (63%) of these emerged from the nest, 350 (20%) were infertile/unhatched eggs, 82 (5%) were root-predated, 22 (1%) were flooded and 54 (3%) were eaten by raccoons despite my nest protectors. Raccoon predation on hatchlings was found in 9 nests in which the predation ranged from 1 to 17 hatchlings. Root predated eggs were found in 19 nests ranging from 1 to 10 eggs in nests. When I excavated nests, I found 25 hatchlings alive in nests and 107 hatchlings dead in nests. There were also 34 eggs that I protected, but when I excavated the nests, I found no indication of what happened to the eggs. I assume that these eggs hatched and emerged from the nest, but escaped before I could properly

measure them. There was never more than one egg missing per nest. Therefore, I used data on 1107 hatchlings for the emergence success analysis. The average number of days before each nest had its first hatchling emerge was 62 ± 8 days ($N = 136$, range = 50 to 90 days). Average nest depth was 14.9 ± 2.2 cm ($N = 136$, range = 9.5 to 20cm). At least some hatchlings emerged from each nest in 2005 by 105 days post-oviposition, and I didn't leave any nests to overwinter.

I ran a backward stepwise multiple regression model comparing the significance of all the microhabitat characteristics, densiometer readings, nest temperature (PTUs and average nest temperature during the TSP) and nest depth, and found that hatchling survivorship was significantly influenced by nest depth ($p = 0.003$, $r^2 = 0.063$) and not by any other variables (Table 10). The model also revealed that depth was significantly correlated with average temperature during the TSP ($r(122) = -0.286$, $p < 0.001$) and PTUs ($r(122) = -0.489$, $p < 0.001$).

Root predation on the eggs was significantly affected by the amount of area around the nest that contained grass. As the amount of grass around the nest increased, the root predation that the eggs experienced increased ($p = 0.036$, $r^2 = 0.032$). Root predation was not significantly affected by the amount of area around the nest that was bare ground ($p = 0.240$, $r^2 = 0.010$), although the presence of grass was negatively correlated with the amount of bare area at the nest site ($r(122) = -0.563$, $p < 0.001$) (Figure 8). Root predation was also not significantly affected by nest depth ($r^2 = 0.009$, $p = 0.274$).

Nest depth was not dependent upon female size ($r^2 = 0.003$, $p = 0.501$), egg size ($r^2 = 0.027$, $p = 0.056$), clutch size ($r^2 = 0.004$, $p = 0.480$) or month laid ($F = 0.035$, $p =$

0.158). Average nest depths were not significantly different between the two years ($\chi^2(1) = 144, p < 0.05$).

Clutch size was dependent upon female carapace size ($N = 137, r^2 = 0.047, p = 0.011$). Average egg size was also dependent upon female carapace size ($N = 137, r^2 = 0.103, p = 0.000$). Emergence success was not dependent upon female carapace size ($N = 134, r^2 = 0.001, p = 0.704$). Emergence success was also not affected by clutch size ($N = 135, p = 0.0813, r^2 = 0.000$) or average egg mass ($N = 135, p = 0.912, r^2 = 0.000$).

In 2005 there were 12 females for which I had data on their earlier and later season clutches. Emergence success, nest depth, clutch size, and average egg mass were not significantly different between the two clutches ($F = 2.477, df = 11, p = 0.144, F = 0.478, df = 11, p = 0.478, F = 0.000, df = 11, p = 1.0, F = 0.722, df = 11, p = 0.414$, respectively).

I obtained data on 14 females which laid nests in both 2004 and 2005. Again, the actual clutch number was unknown. Neither emergence success, nest depth, clutch size, nor average egg mass differed significantly between the two years ($F = 0.46, df = 13, p = 0.833, F = 0.001, df = 13, p = 0.980, F = 2.812, df = 13, p = 0.117, F = 1.090, df = 13, p = 0.315$, respectively).

Compared to 2004, significantly more eggs were desiccated in 2005 ($N = 350, 20\%$) than in 2004 ($N = 75, 4\%$) ($\chi^2(1) = 180.89, p < 0.05$), more hatchlings were found dead in the nest in 2005 ($N = 107, 6\%$) compared to 2004 ($N = 30, 1.6\%$) ($\chi^2(1) = 53, p < 0.05$) and more hatchlings were found which had broken through the egg shell but failing to emerge from the egg completely in 2005 ($N=19, 1.12\%$) compared to 2004 ($N = 0, 0\%$) ($\chi^2(1) = 19.7, p < 0.05$).

On average nests hatched much quicker in 2005 (76 days) than in 2004 (62 days); this difference was significant ($t(242) = 12.153, p < 0.001$). There was no significant difference found in average nest depth between the two years ($t(278) = 1.132, p = 0.259$).

DISCUSSION

Terrapin nesting ecology at Jamaica Bay Wildlife Refuge in 2004 and 2005 was generally similar to reports from this site in previous years (Feinberg and Burke 2003; Ner 2004; Giambanco 2004) and other sites in the same region (Burger 1977). However, during the warm, wet summer of 2005 the average incubation period was significantly shorter (62 days) than in 2004 (76 days). Previous studies on terrapins have shown that the average incubation time increases with decreasing latitude; average incubation was 68.9 days in northeastern Florida (Butler et al. 2004), 54.5 days in South Carolina (Zimmerman 1992), 76 days in New Jersey (Burger, 1977), 73.7 days in Rhode Island (Goodwin 1994) and 108 days in Massachusetts (Auger and Giovannone 1979). The 2005 incubation period at JBWR was closer to the average incubation period for terrapins in South Carolina than it is to the incubation period for 2004. Therefore I examined my results from the different years separately.

Overall, emergence success (eggs which successfully hatched and hatchlings emerged from the nest) at JBWR (70% in 2004, 63% in 2005) was similar to previous studies. Burger (1977) found the emergence success of nests in New Jersey to be 69%. There have been three previous relevant studies at JBWR. Cook (1989) found that emergence success in the early 1980s (before raccoons became common at JBWR) was 93% (N = eight nests), Feinberg (2000) found emergence success at nests not predated by

raccoons in 1999 to be 57% (N = 3), and Giambanco (2003) reported emergence success of unpredated nests as 88.6% in 2000 (N = 68) and 86.5% in 2001 (N = 61).

The two nests I documented over-wintering in 2004 appear to be the first documented occurrence of diamondback terrapins over-wintering in the nest in New York. I could not determine whether the overwintering I observed was as eggs or hatchlings because I did not excavate the nests until hatchlings emerged. Hatchlings have previously been documented overwintering on land after hatching in New York (Draud et al. 2004). Hatchling diamondback terrapins are freeze tolerant (Costanzo et al. 2006), and Baker et al. (2006) showed that in New Jersey, diamondback terrapin hatchlings were likely to over-winter in the nest 50% of the time, which involved being exposed to subfreezing temperatures, and 87% survived winters in the nest. Baker et al. (2006) also revealed that hatchlings would be more successful if none emerged in the fall and all waited to emerge in the spring. Although I only allowed eight nests to overwinter, it is interesting that none of the nests with fall emergence also had spring emergents. One benefit of hibernating inside the nest could be a reduction in exposure to predators during a time when food resources necessary for rapid growth are in decline (Baker et al. 2006). Another benefit for overwintering in the nest could be the ability to enter an environment with abundant food resources (spring) as opposed to an environment in decline (fall) (Gibbons and Nelson 1978). One reason that we see so little overwintering in the nest at our study site could be due to the fact that most of our nests are laid in sand, which is a porous and coarse soil and is very susceptible to inoculative freezing, which would cause many hatchlings to die (Baker et al. 2006).

Emergence success appeared to be dependent upon more than one environmental factor and these differed between the two study years. In 2004, emergence success was dependent upon the percent grass found in the vicinity of the nest, the average nest temperature during the TSP and the densiometer readings from the East. The percent grass also strongly affected nest temperature in 2004 (chapter 2). Densiometer readings (solar exposure) have also proven to be important when females are choosing their nest sites (chapter 1).

In 2005, nest depth appeared to have the largest effect on hatchling survivorship, probably because ambient temperatures in 2005 were so high (Chapter 2). This suggestion is bolstered by the observation that nest depth was significantly associated with nest temperature of the nests in 2005 and 2004 (see chapter 2), in that deeper nests were cooler.

Interestingly emergence success was not dependent upon female body size in either year. Larger females did not have greater fitness (reproductive success). It has been found that hatchling turtles benefit more from being large by having increased fitness (Janzen et al. 2000), but this does not seem to hold true in this adult population of terrapins because larger females did not have greater reproductive success. These results are most likely due to the fact that turtles have no maternal care and the incubation environment affects hatchling phenotypes. Because of this female nest site choice is one of the most determining factors affecting emergence success.

Because female terrapins can lay 1-3 nests per year at my study site (Feinberg and Burke 2003), it is unknown which clutch of eggs the nests were from. This may also

have an impact on emergence success, but this was unable to be measured during this study.

In both 2004 and 2005 I found that as the percentage of area covered by grasses increased, hatchling survivorship decreased. But, only in 2004 was grass the most important factor for determining emergence success. These results help explain previous results (Chapter 1) that nesting terrapins selectively avoid grassy/shrubby microhabitats for nest site selection. Grass avoidance is crucial because grass roots can reach deeper than a meter (Burke, pers. obs.), so it is impossible for females to attempt to avoid grass roots by digging a deeper or shallower nest. This strategy of grass avoidance can be helpful to females because grass roots predate terrapin eggs (Lazell and Auger 1981; Feinberg and Burke 2003; Giambanco 2004; Stegmann et al. 1988) and because the presence of grasses decrease the temperature of the nest (chapter 2).

Nest temperature, measured in PTUs, was not a significant factor affecting hatchling survivorship in 2004 and 2005. But in 2004 (not 2005), hatchling survivorship was significantly affected by the average temperature during the thermosensitive period. The fact that nest temperature was not a significant determining factor of hatchling survivorship in 2005 is very intriguing. Average nest temperatures and PTUs were both significantly and negatively correlated with nest depth, so that the deeper the nest, the lower the nest temperatures and the PTUs. It may be that because nest temperatures were so high, hatchlings were doomed in shallow nests and therefore nest depth played the largest role in determining hatchling survivorship. Giambanco (2004) found that under constant incubation temperatures, terrapin eggs from JBWR had >95% hatchling survivorship at 26°, 28°, 30°, and 32°, but dropped to 80% at 34° C. It is interesting that

in 2004, depth had the opposite effect on hatchling survivorship than in 2005. In 2004, the deeper the nest, the less likely it was for the hatchlings to emerge. This is most likely due to the deeper eggs experiencing too low of temperatures to develop properly. This has also been shown to happen to terrapins in New Jersey (Burger 1977).

In the summer of 2004, when the temperatures were close to typical for the region, emergence success was determined by the amount of grass around the nest and the overhead cover. Therefore hatchlings had a better chance of surviving if they were in a nest that was not near grasses or other vegetation. In 2005, emergence success was strongly influenced by the nest depth, because in that year deeper nests were cooler and had higher emergence success. Many more eggs were desiccated in 2005 than in 2004 and there were also many more hatchlings that died in the nest in 2005. But, unless females can accurately predict how ambient temperature will increase over a summer and dig their nests accordingly, this represents a shifting nesting environment with no clear optimum. And, the results of this study show on average females did not dig nests of different depths in the two years. But, in 2004, there was a significant difference in nest depth between early and late clutches, so the rationale of why some females lay deeper nests may be important to be looked at in future studies.

Nest depth and nest temperatures affected the hatchling survivorship in different ways in the two different years, supporting the hypothesis that greater nest depth can ameliorate the effects of extreme temperatures. Nesting in a more highly vegetated area, as opposed to the open sand, could also shield the nest from extreme temperatures. But nesting near vegetation can have detrimental effects on eggs through nest predation by plant roots.

CONCLUSION

My research was conducted during two summers with very different climatic patterns, which clearly affected nest conditions and emergence success. In 2004, a climatically typical year, grass cover was an important factor affecting both nest temperatures and emergence success. But grass cover was not one of the factors used by female terrapins to determine where to place their nests; instead, they used solar exposure.

In 2005, an unusually warm and dry year, it is apparent that there are different mechanisms affecting nest temperature and emergence success rate. Females appear to use solar exposure, the presence of bare ground, and litter coverage in nest site selection. These factors are plausible choices for females to use based on their ability to recognize them. But nest temperatures and emergence success were actually determined by nest depth, presence of bare ground and overhead cover.

My research shows that females most likely choose nest sites based on both solar and vegetation cues, and that these cues affect nest temperatures and hatchling survivorship differently based on annual climatic variation. These results also show the impact of fluctuating nest temperatures on the nest success of female terrapins. Although I did not determine the sex of these hatchlings, it can be inferred from the temperature data that the majority of nests laid in 2005 were most likely be female-biased. Climatic models for the study region (Rosenweig and Solecki 2001) predict that temperatures and rainfall will increase over the next century. Therefore my results indicate selection pressure for deeper nests in sunny areas or nesting in more vegetated areas in order to

protect eggs from desiccation or an overabundance of female-biased nests during warmer nesting seasons.

ACKNOWLEDGEMENTS

I would like to thank the Hudson River Foundation for awarding me a Polgar Fellowship, which enabled me to afford so many temperature loggers. The Department of Biology of Hofstra University provided essential financial support through a Donald A. Axinn Fellowship. My advisor, Dr. Russell Burke, was very helpful both in the field and while writing this paper. His never-ending support kept me going through the writing process. Dr. Christopher Sanford was extremely helpful with the statistical analysis in this paper.

Chris Olinjynk of the National Park Service was very helpful with equipment set up and removal and allowing me to use his GPS equipment. Asma Madad helped considerably by assisting in the sexing of the hatchlings. Eric Rulison also helped considerably by watching my terrapins, nests and hatchlings while I was away. Beth McGuire also helped by looking out for my hatchlings and making sure they always had water when they needed it. I would like to thank Sylwia Ner for teaching me how to hunt for terrapins and how to pit-tag.

Also I would like to thank all of my volunteers. Michael Unold took many great photographs. Many volunteers, high school students and Hofstra University undergraduate students helped with this project. But, I would especially like to thank Avi Lewis and Barbara Wasserman who helped out greatly over the past two years whether it was with checking nests while I was away or just being there every Saturday and more to help out with the project. Barbara Trees and Amaury Aquines were also excellent

volunteers who were always there when needed. Without such great volunteers I would never be able to have such a large sample size of nests.

Most of all I would like to thank my husband, Dustin, for his support through this process. Dustin helped to collect field data and also helped to analyze a lot of the temperature logger data. Most of all, he was there for support in times when I needed reassurance and encouragement to finish this thesis.

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Figure 1. Study site: Jamaica Bay National Wildlife Refuge



Figure 2. Nest depth frequency distribution in 2004.

2004 Nest Depth Distribution

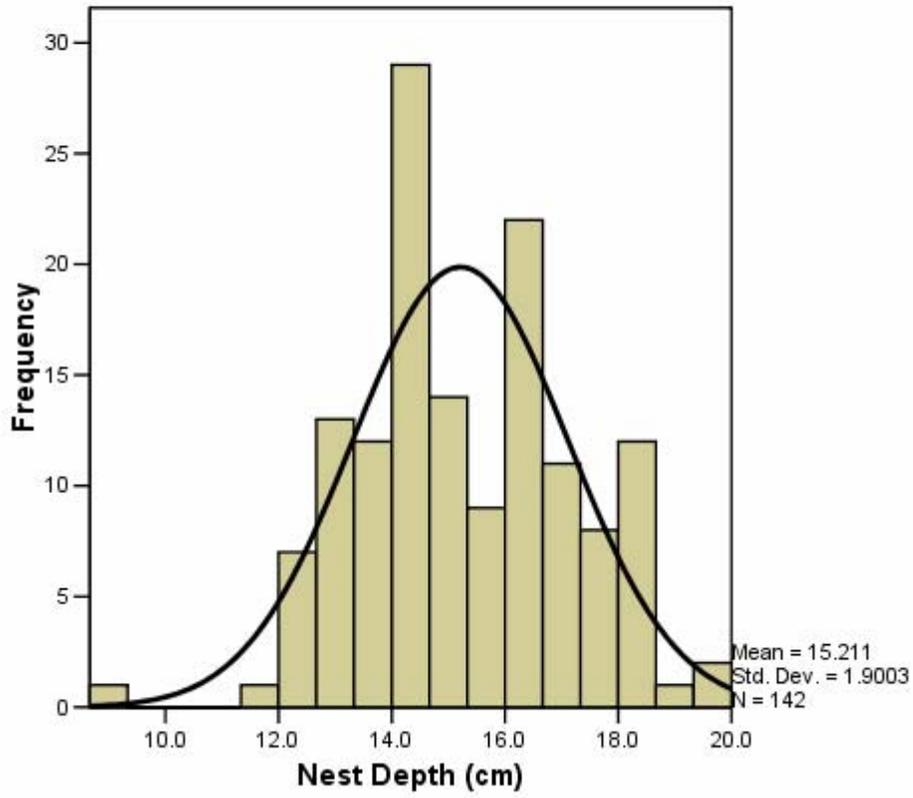


Figure 3. PTU (pivotal temperature units) frequency distribution in 2004.

PTU Distribution in 2004

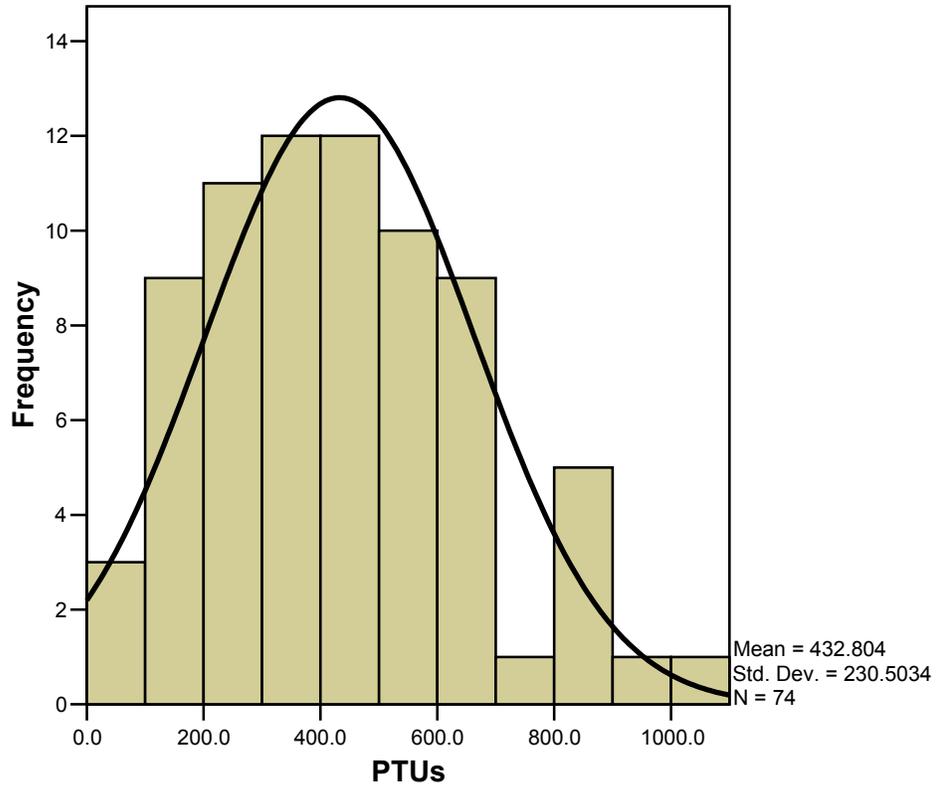


Figure 4. Frequency distribution of average nest temperature during the TSP for 2004.

2004 Average TSP Nest Temperature

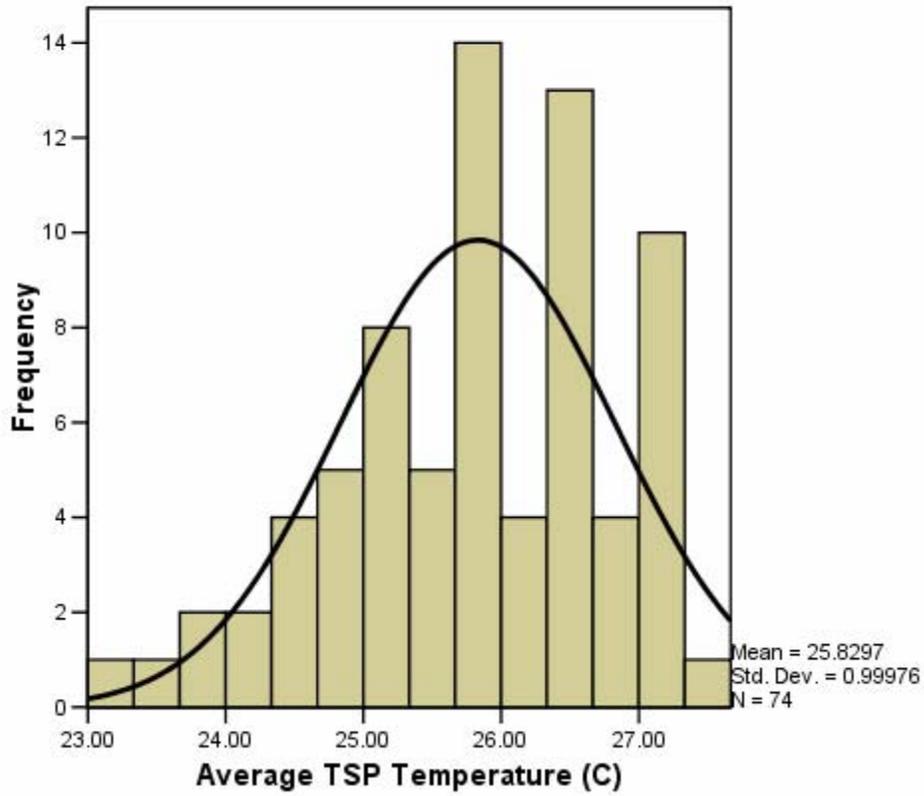


Figure 5. Nest depth frequency distribution in 2005.

2005 Nest Depth Distribution

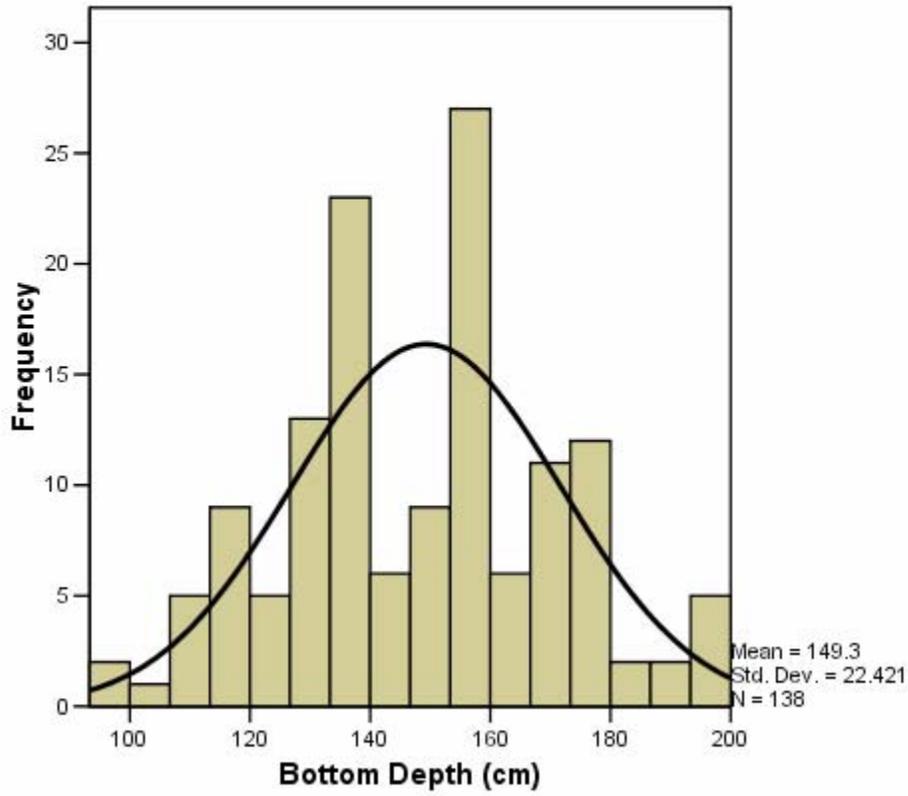


Figure 6. PTU (pivotal temperature unit) frequency distribution in 2005.

PTU Distribution in 2005

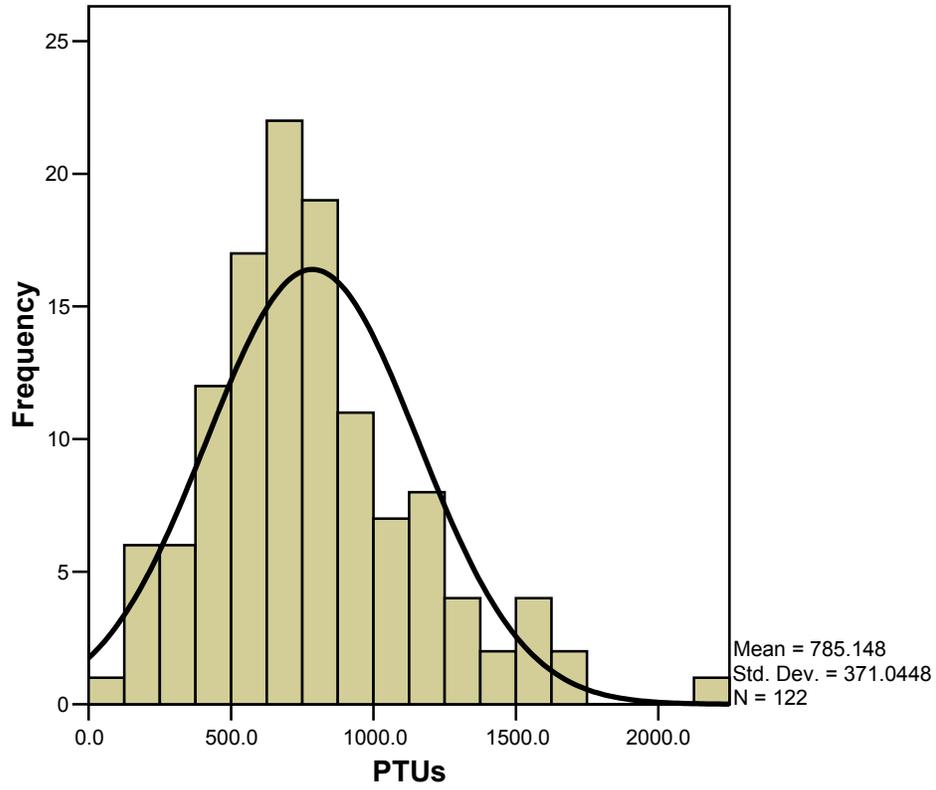


Figure 7. Frequency distribution of average nest temperature during the TSP in 2005.

2005 Average Nest TSP Temperature

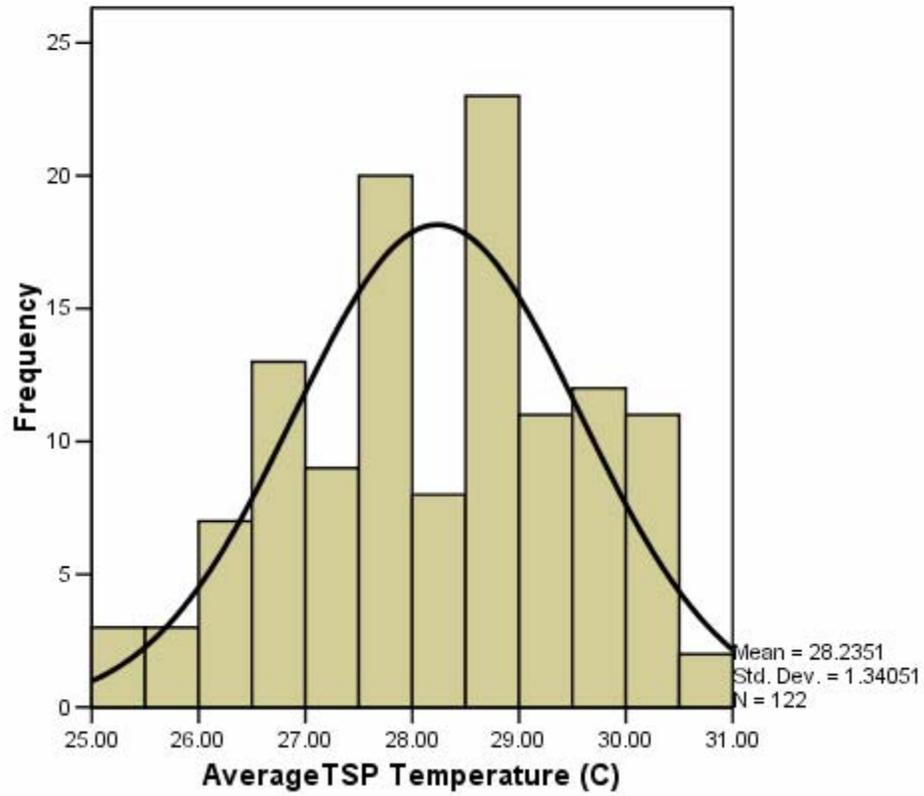


Figure 8. The percent bare ground compared to the percent grass in 2004.

% Bare vs. % Grass 2004

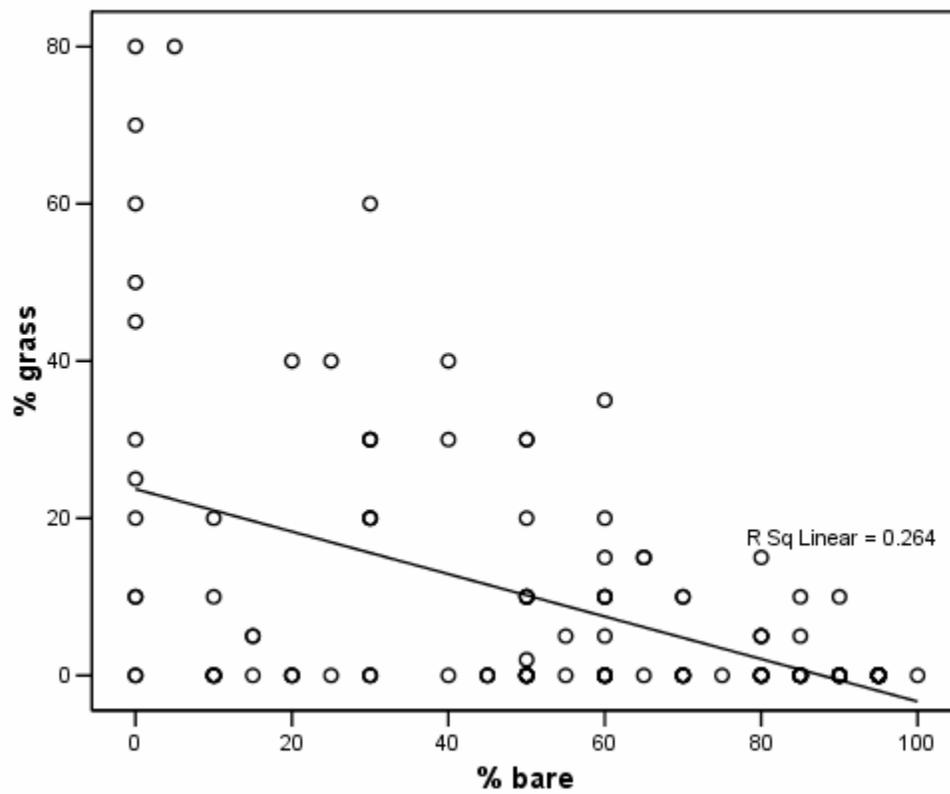


Figure 9. The percent grass compared to the percent bare ground in 2005.

% Grass vs. % Bare Ground in 2005

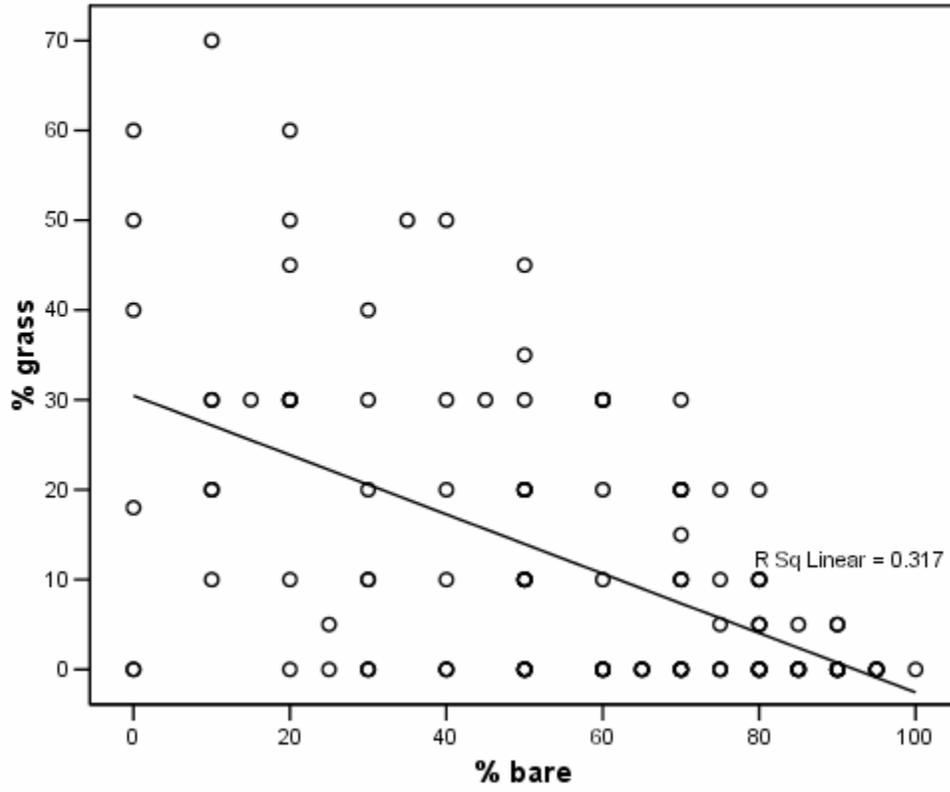


Figure 10. Nest exclosure example from 2004.



Figure 11. Nest exclosure example from 2005.



Figure 12. The effect of nest depth on PTUs in 2004.

Nest Depth vs. PTUs in 2004

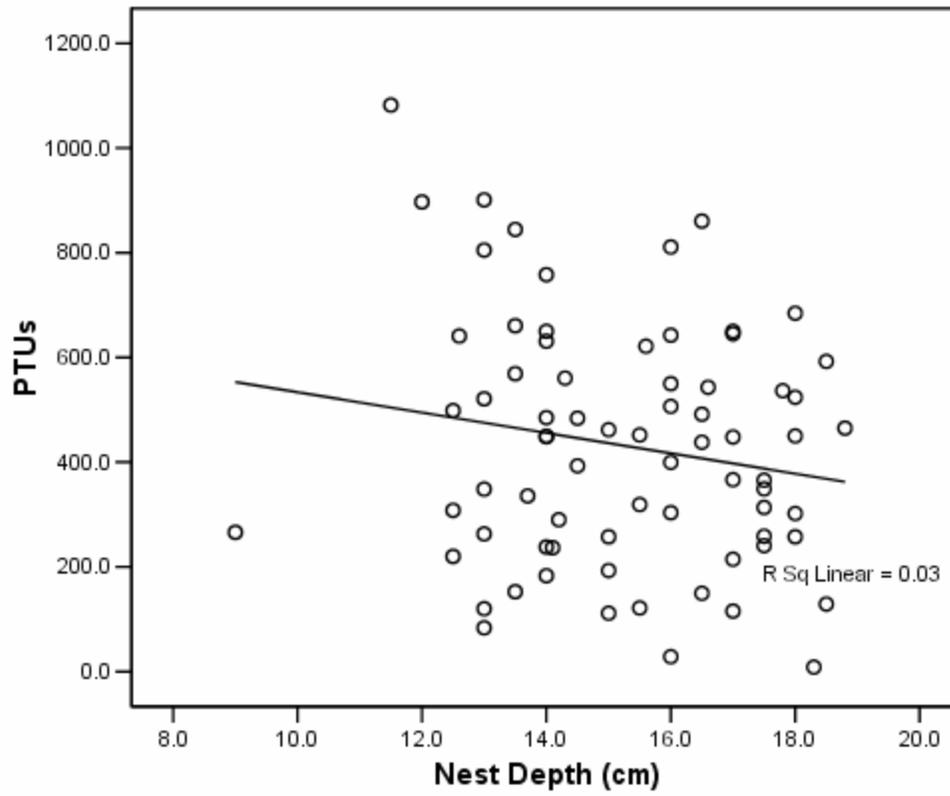


Figure 13. The effect of nest depth on average nest temperature during the TSP in 2004.

Nest Depth vs. Average TSP Temperature in 2004

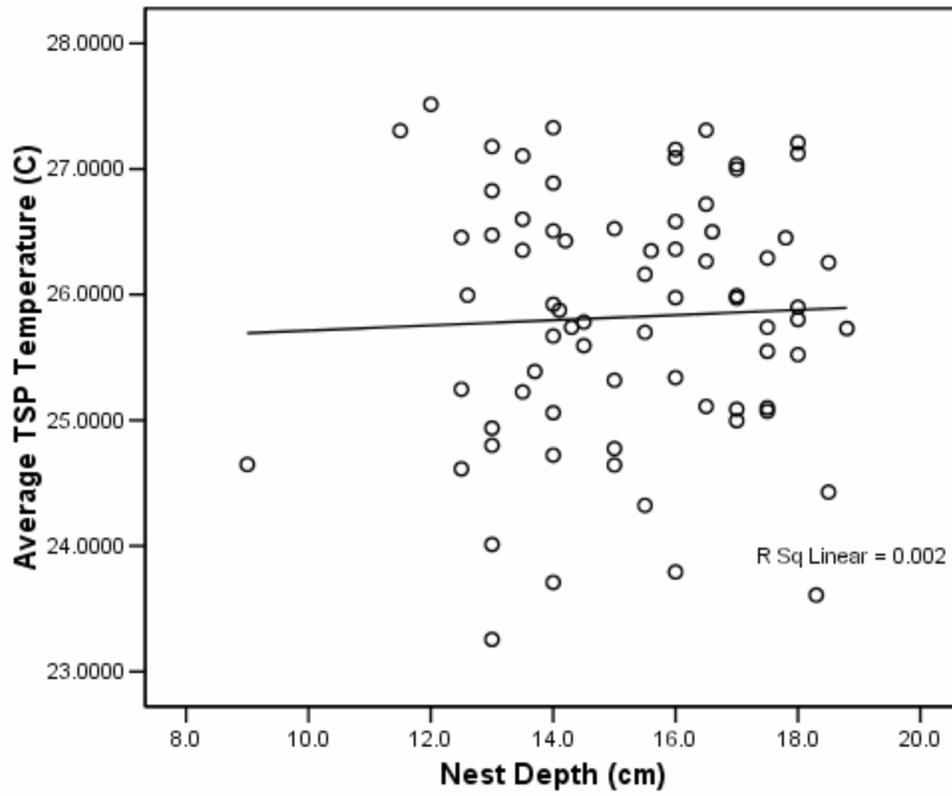


Figure 14. The effect of nest depth on PTUs in 2005.

Nest depth vs. PTUs in 2005

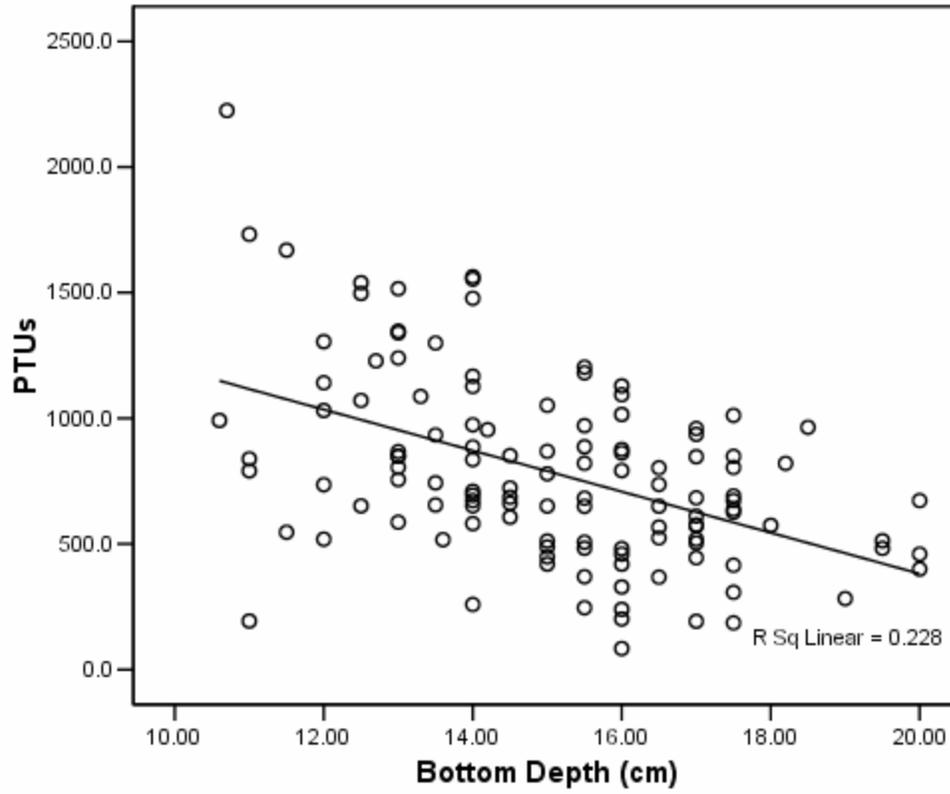


Figure 15. The effect of nest depth on average nest temperature during the TSP in 2005.

Nest Depth vs. Average TSP Temperature in 2005

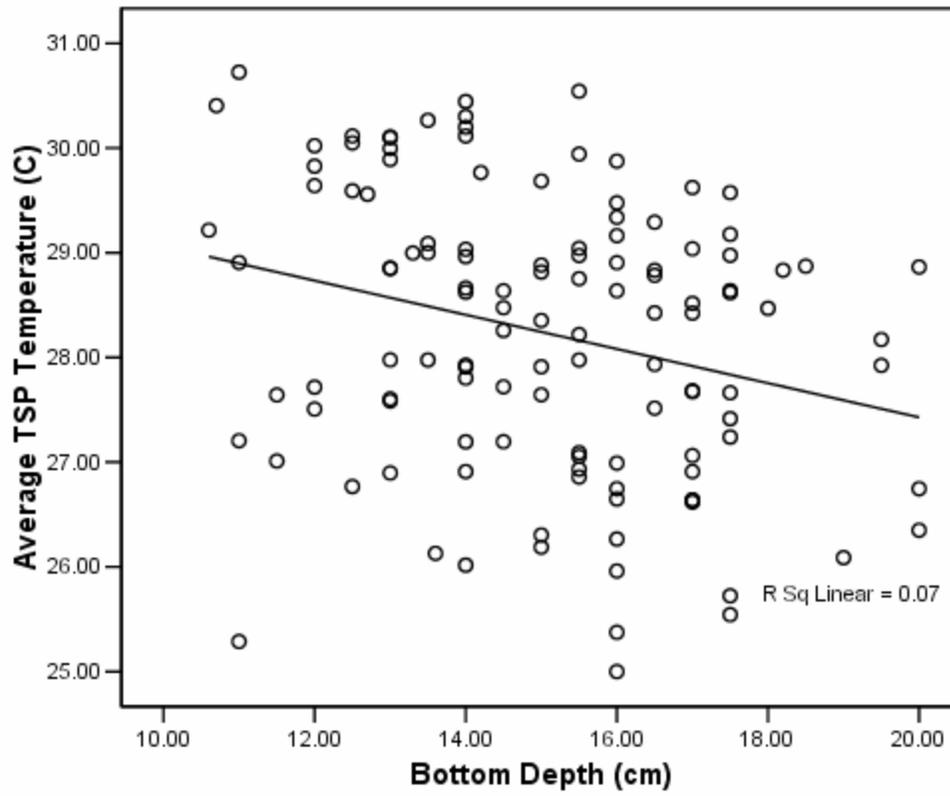


Table 1. Results of binary logistic backward stepwise multiple regression analysis used to determine how nest sites differed from random in 2004. The reduced model of main factors is based on the removal from the larger model of main factors yielding P values of greater than 0.1. Nest sites differed from random by having less overhead cover from the West.

Variable	d.f.	<i>P</i>	S.E.
Densimeter West	1	0.000	0.020

Table 2. Results of binary logistic backward stepwise multiple regression analysis used to determine how nest sites differed from random in 2005. The reduced model of main factors is based on the removal from the larger model of main factors yielding P values of greater than 0.1. Nest sites differed from random by having less overhead cover from the North and South and more bare ground and less litter.

Variable	d.f.	<i>P</i>	S.E.
Densiometer North	1	0.055	0.030
Densiometer South	1	0.000	0.034
% Bare Ground	1	0.028	0.004
% Litter	1	0.000	0.010

Table 3. Backward stepwise multiple regression results of microhabitat effects on average nest temperature during the thermosensitive period in 2004. The reduced model of main factors is based on the removal from the larger model of main factors yielding P values of greater than 0.1. The percent grass around the nest site was the most determining factor of nest temperature during the thermosensitive period in 2004. The slope shows the correlation between the independent and dependent variable.

Variable	d.f.	N	<i>P</i>	r^2	Slope
% Grass	1	74	0.039	.060	-

Table 4. Multiple regression results of microhabitat effects on nest critical thermal units (PTUs) during the thermosensitive period in 2004. The reduced model of main factors is based on the removal from the larger model of main factors yielding P values greater than 0.1. PTUs were most influenced by the percent grass and dicotyledonous plants around the nest and the nest depth in 2004. The slope shows the correlation between the dependent and independent variable.

Variable	d.f.	N	<i>P</i>	r^2	Slope
% Grass	1	74	0.008	0.080	-
% Dicots	1	74	0.047	0.016	-
Nest Depth	1	74	0.055	0.028	-

Table 5. Backward stepwise multiple regression results of microhabitat effects on average nest temperature during the thermosensitive period in 2005. The reduced model of main factors is based on the removal from the larger model of main factors yielding P values greater than 0.1. Average nest temperature was most determined by the nest depth, percent bare area around the nest and densitometer readings from the North, South and East in 2005. The slope shows the correlation between the dependent and independent variable.

Variable	d.f.	N	<i>P</i>	r^2	Slope
Nest Depth	1	122	0.001	0.082	-
Densitometer North	1	122	0.001	0.054	-
Densitometer South	1	122	0.085	0.003	-
Densitometer East	1	122	0.043	0.008	-
% Bare area	1	122	0.095	0.008	+

Table 6. Backward stepwise multiple regression results of microhabitat effects of nest critical temperature units (PTUs) during the thermosensitive period in 2005. The reduced model of main factors is based on the removal from the larger model of main factors yielding P values greater than 0.1. PTUs were most influenced by nest depth in 2005. The slope shows the direction of the correlation between the independent and dependent variable.

Variable	d.f.	N	<i>P</i>	r^2	Slope
Nest Depth	1	122	0.0000	0.239	-

Table 7. Results of nesting success in both 2004 and 2005.

	2004	2005
Average days to 1 st emergence	76	62
Total # of eggs	1852	1720
Hatched Eggs	1299	1078
# of dead eggs	75	355
# of root-predated eggs	97	82
# of raccoon-predated eggs	265	54
# Live hatchlings in nest upon excavation	154	25
# of dead hatchlings in nest upon excavation	0	19
# of hatchlings dead-pipped	30	88
# of eggs flooded	110	22
# of eggs unaccounted for	95	34

Table 8. Backward stepwise multiple regression results of microhabitat effects including PTUs, average nest temperature during the TSP and nest depth on hatchling emergence success in 2004. The reduced model of main factors is based on the removal from the larger model of main factors yielding P values greater than 0.1. Hatchling survivorship was most influenced by the amount of grass around the nest site and the overhead cover to the East in 2004. The slope shows the direction of the correlation between the independent and dependent variable.

Variable	d.f.	N	<i>P</i>	r^2	Slope
% Grass	1	72	0.010	0.063	-
Densimeter East	1	72	0.024	0.016	-
Average nest temperature during the TSP	1	72	0.003	0.119	+

Table 9. Multiple regression results of microhabitat effects including pivotal temperature units (PTUs) during the thermosensitive period, average nest temperature during the thermosensitive period and nest depth on hatchling emergence success in 2005. The reduced model of main factors is based on the removal from the larger model of main factors yielding P values greater than 0.1. Hatchling survivorship was most influenced by nest depth in 2005. The slope shows the direction of the correlation between the dependent and independent variable.

Variable	d.f.	N	<i>P</i>	r^2	Slope
Nest Depth	1	121	0.003	0.071	+