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Intel Project: Nest Site Microhabitat Influences Nest Temperature and Offspring Sex Ratio of the
Diamondback Terrapin (*Malaclemys terrapin*)

I sat as still as the humid air around me, on soft yellow sand lightly punctuated by pebbles

and twigs. With my eyes, I followed an advancing motion that was shaking the lowest branch of

one of the bushes that surrounded my little island of sand. Slowly and deliberately the motion

progressed forward until, upon reaching the threshold where the protection of the leaves ended, it

stopped. A minute or so later, an elaborately patterned green head emerged from the leaves and

two intense black eyes focused on my surroundings and me. I thought I could not remain still

any longer, but from the shadow surrounding the pair of dark eyes, a diamondback terrapin

(*Malaclemys terrapin*) finally took shape and proceeded to gradually make her way over the sand

and pebbles, occasionally stopping to prod into the sand with her nose, dig a small hole, and

move on to another spot, where she repeated her pre-nesting procedure. What was she looking

for? After four test holes, this mother-to-be chose a spot to her liking in a sandy plot near a

small bush and committed her clutch of eggs to the sand and microhabitat. Here they would

incubate from seventy to one-hundred days. Yet why did she make her nest in this particular

spot, near this particular bush?

I got up, looking at the Empire State Building and the city I grew up in, which seemed to

rise from the marsh in the distance, and wondered what a mother turtle could do for her offspring

before she disappeared into the murky waters of Jamaica Bay Wildlife Refuge. New York City

is a challenging place to grow up if you are a terrapin. Even before you hatch, there is a very

good chance that a raccoon may dig up the nest you are peacefully incubating in and devour you

along with your brothers and sisters (Feinberg and Burke 2003). If you are incubating in a nest

close to dense grass, the roots may penetrate your eggshell and feed off of the yolk, leaving

nothing in your eggshell besides a tangled up roots (Lazell and Auger 1981; *pers. obs.*) (Figure 1). If you survive long enough to emerge from your nest and make it past the predators on land, a new set of threats await you in the waters of the estuary, including barnacle infestation (Seigel 1983) and the possibility of drowning in crab pots (Roosenburg et al. 1997; Wood 1997). I had seen many terrapin eggs fall victim to raccoons and grass as I conducted a study on nest site selection during the 2003 nesting season. I compared the microhabitat variables, including the overhead canopy cover of a nest and the percent of bare ground, litter, dicotyledonous plants, and grass in a one-meter plot around the nest, of real terrapin nests to those of randomly chosen spots using a χ^2 test. I saw that significant differences existed between all microhabitat characteristics of terrapin nests and random points, except the percent of grass. This statistical test revealed that terrapins chose their nest sites based on microhabitat variables. The amount of grass at nest sites compared to random points was not significantly different probably because so few nests are laid in areas of dense grass. This introduced the possibility that nesting terrapin females could be avoiding areas of high grass densities to prevent their offspring from being gripped by grass roots and dying a slow and inescapable death. Consequently, grass became a possible criterion for nest site selection, a factor based on which terrapins chose nest sites. Whether the other microhabitat variables were criteria remained a mystery. Most nests were laid in open areas with little overhead canopy and modest quantities of vegetation and litter (Wojakowski and Burke 2003). Why?

Scientists are very familiar with diamondback terrapin reproductive biology (Roosenburg 1994; Burger and Montevecchi 1975; Burger 1976; Burger1977; Seigel 1980; Feinberg and Burke 2003). My first nesting season in 2003 progressed and their discoveries unfolded before my eyes. Most terrapins emerged each day with the high tide and each laid a clutch of about 13

eggs (Feinberg and Burke 2003). Even while gathering the data that would ultimately show terrapins in Jamaica Bay Wildlife Refuge had nest site selection, I saw what Roosenburg (1994) meant when he called terrapins "opportunists" that "specialize in the requirements of nest sites" as I watched nesting females dig test hole after test hole. Yet nest site selection is only one piece of the terrapin nesting puzzle. The pieces identifying the reasons for selection had still to be discovered and put together.

Scientists in laboratory experiments have shown that terrapins have temperature-dependent sex determination (TSD) (Jeyasuria et al. 1994). Organisms with genotypic sex determination (GSD), like humans and certain turtles, including soft-shelled turtles *Apalone spinifera* (Bull and Vogt 1979) and mud turtles *Kinosternon baurii* (Sites et al. 1979), have sex chromosomes. Their offspring have a 50% chance of being male and a 50% chance of being female. The sex of offspring of organisms with TSD, however, is determined by incubation temperature. In laboratory studies using a constant incubation temperature, it has been determined that terrapin clutches incubating at temperatures higher that 29°C produce mostly females, while those incubating at temperatures below 29°C produce males (Jeyasuria et al. 1994). However, TSD in terrapins had not been studied in the field, where temperature fluctuates. Perhaps it was a missing piece of the nest site selection puzzle, as western painted turtle (*Chrysemys picta belli*) researcher Fredric Janzen (1994) suggested when he explained that painted turtles may use vegetation around a nest site to predict the nest temperature and sex ratio of their offspring. This would be the criterion for choosing a nest site. In addition, it would allow the nesting female to not only control the sex ratio of her offspring, but also their fitness, or their ability to survive and compete for resources. This hypothesis is very reasonable because turtles seem to be the strongest exhibitors of TSD, and it has been shown that nests incubating in optimal conditions

usually result in single-sex clutches, reducing inbreeding (Burke 1993) and increasing survivorship (Janzen 1995; Seigel 1980).

Vegetation seemed to be a logical indicator of nest temperature for nesting females, and perhaps this was the factor nesting terrapin females considered when selecting their nest sites. In a population threatened by raccoons, humans, and even grass, the nesting female's control over hatchling sex and fitness would be beneficial to her. Looking at hatchlings as they crawled away from nests and headed straight for the nearest source of shade, I thought that a hatchling's ability to identify shade signaled the same aptitude in an adult terrapin. Consequently, I too hypothesized that, if TSD existed in the field for diamondback terrapins, vegetation was the factor by which terrapins chose nest sites. If a turtle exhibited TSD in the field, a correlation would exist between nest microhabitat, nest temperature, and sex ratio (Janzen 1994). Therefore, I hypothesized that this correlation exists in the field for terrapins and the vegetation influences nest temperature, which influences the sex ratio of the offspring.

I conducted the study during the 2004 nesting season in Jamaica Bay Wildlife Refuge. Terrapin females are so skilled at concealing their nests, that it is virtually impossible to identify a nest without seeing it laid or without visible tracks pointing to its location. Even after watching a female nest for twenty minutes, it is often difficult to correctly identify the location of the cavity after she has walked away. As a result, all nests used in the study were made by females which were observed nesting by students, volunteers, and myself. Once the female was on her way back to Jamaica Bay, each nest was excavated and the eggs removed in the order they were laid. A temperature logger was placed at the bottom of the nest cavity, the eggs were replaced in the order they were laid, and another temperature logger was placed at the top. These temperature loggers would record the nest temperature hourly for 100 days. If the incubation

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period extended past 100 days, the loggers would record over the initial readings until stopped. Each nest was covered with a flat, standard ½ inch hardwire cloth in the shape of a square 61 x 61 cm^2 and fastened down with stakes to serve as protection from raccoon predation. The habitat and location of each nest were recorded and the overhead canopy cover measured with a spherical densiometer (Figure 3). Then the percent of bare ground, litter, dicotyledonous plants, and grass were estimated for a one-meter plot around each nest and recorded. Fifty days after oviposition, the flat hardwire cloth was replaced with a 2.5 cm tall box-shaped cloth of the same dimensions in order to give the hatchlings room to emerge. From this point on, the nests were checked everyday, at least once daily. When hatchlings did emerge, they were removed from within the hardwire cloth and each hatchling's carapace length and tail length were measured with a 6-inch dial caliper. After measurement, the hatchlings were immediately released. The measurements of carapace and tail length (Figure 4) were used to determine hatchling sex according to a technique developed by Giambanco (2002) and the sex ratio of the entire clutch was expressed as the percent of males for the purpose of analysis.

Data from 100 nests were used in the statistical analysis. Since hatchling sex is determined during the thermosensitive period (TSP), which in terrapins is the middle third period of incubation, I isolated the TSP from the complete temperature data set of the top loggers of every nest. The results of an ANOVA showed mean nest temperature was highly related to sex ratio, and that CTUs (Critical Temperature Units, or the number of readings above the 29° sex determination threshold) were highly related to sex ratio as well. These significant relationships showed that terrapin hatchling sex in the field is determined by nest temperature. This was the evidence necessary to conclude that diamondback terrapins have TSD in the field, and the highly

significant relationship between CTUs and sex ratio showed how important temperature fluctuations that exist in the field, but not in the laboratory, are in sex ratio determination.

Further statistical analysis showed that TSD is not independent of the nest microhabitat. Regression tests showed that all microhabitat variables are related to the mean nest temperature except the percent of dicotyledonous plants at a nest site. This is not surprising, since dicotyledonous plants are seasonal. This variability throughout the nesting season is amplified by their substantial surface area. Plants that change so much may not have a dramatic impact on mean nest temperature, a measurement of stability. However, they have a strong impact on a measurement of variability, CTUs. In fact, ANOVAs and regression tests showed that all microhabitat variables have an impact on CTUs, but especially the percent of bare ground (Figure 5) and grass (Figure 6). These results show that hatchlings are sensitive to repeated extremes in temperature generated by microhabitat interaction with environmental components, such as solar intensity and wind. The diversity of physical properties of the vegetation and litter of a nest site, and the way in which vegetation and litter respond to different intensities and directions of wind and rain, creates the complex and perpetually shifting geometric configuration with which solar rays interact to influence both the ambient temperature and nest temperature of a site. The significant impact of grass on CTUs may result from its small surface area and extent to which it can be manipulated by wind or rain, while bare ground, devoid of vegetation, quickly reacts to changes in solar intensity.

The research showed that microhabitat plays an instrumental role in TSD in the field, indicating that nesting terrapins could predict sex ratios based on microhabitat under one condition. Terrapin females would need to be able to predict an average nest temperature and thermal extremes, often caused by storms and even hurricanes as far away as Florida, some thirty

days into the future. While terrapins may have this amazing skill, it is more likely that their choice of nest site is determined by conditions the day of nesting. If terrapins truly do have control over the sex ratio of their offspring, their decisions are probably based on a measurement of temperature that can be estimated and predicted with more accuracy than thermal extremes. Their decision would be based on a more stable measurement, such as an average. This could be evidence that the TSD mechanism is adapted to nest site selection. Yet mean nest temperature is a measurement of stability not restricted to TSP. Mean nest temperature during TSP ought to be very similar to that of the entire incubation period. If a correlation existed between nest microhabitat, mean nest temperature, and sex ratio for the complete incubation period, it would be evidence that a terrapin's prediction of temperature based on microhabitat could indeed allow her to control her sex ratio. Furthermore, it would identify the microhabitat factor(s) that terrapins used to choose nest sites in order to control sex ratios.

As a result, I turned to the analysis of the complete temperature data set. During the hatching season, it had become evident that incubation time and emergence time for terrapin hatchlings were not the same. Often hatchlings remained in nests after hatching. For several nests, emergence times exceeded 100 days, and the temperature loggers began to record temperature measurements over the initial temperature measurements. When used in the analyses, the data from these loggers would add error. Therefore, if significant relationships existed, they would be the strongest relationships possible in the nest site selection process. In this case, the small error would add an additional degree of certainty.

Regression analyses showed that mean nest temperature is related to sex ratio, and related to the percentage of grass and bare ground around a nest site. These results indicated that grass could be used to predict nest temperature during nest site selection, but my previous study

showed that relatively few nests are laid in areas of high grass densities. Therefore, grass may not be a common temperature indicator, leaving bare ground, not vegetation, as the most likely indicator of nest temperature for nesting terrapins. This may explain why the majority of nests in Jamaica Bay Wildlife Refuge are located in areas of mostly bare ground with fairly small quantities of vegetation (Wojakowski and Burke, 2003). Additionally, it explains why the regression test showed that overhead canopy cover was not related to mean nest temperature for the complete temperature data set. Furthermore, bare ground is a dependable predictor of nest temperature because, unlike any form of vegetation or litter, it is the microhabitat variable most likely to remain constant from oviposition to hatching.

In his study of painted turtles in Illinois, Janzen (1994*b*) found that vegetation cover was the nest site variable that remained most stable from oviposition to hatching, influencing both temperature and sex ratio. However, the results showed that, for diamondback terrapins, the more important factor is bare ground. Since a terrapin's sight is less acute on land than in the water, it may be easier to identify a bare plot than to distinguish vegetation from other objects. Hatchlings crawling away from a nest could easily identify shade, but many times were unable to make the distinction between vegetation and other structures. If this is characteristic of adult terrapins, then a bare plot is the most accurate indicator of nest temperature because an absence of vegetation eliminates the question of whether a shade giving structure is a plant. Furthermore, absence of vegetation includes absence of grass, meaning that the developing hatchlings will not be exposed to the risk of grass predation.

While relationships existed between microhabitat, nest temperature, and sex ratio for the complete temperature data set, a significant relationship did not exist between nest microhabitat components and sex ratio. This shows that a terrapin female cannot predict her sex ratio based

on microhabitat alone. When a female comes on land to nest, it appears she chooses a nest site based on the amount of bare ground. Microhabitat components have a direct effect on the incubation temperature. As a result of the incubation temperature, a certain number of male and female hatchlings develop and emerge. One variable triggers the next in a sequence of specific order, ultimately resulting in a sex ratio. In this way, the negative result for TSD is that sex ratio is a direct product of microhabitat. This reproductive and developmental process indicates a deliberate series of cause and effect events initiated by the female's choice of nest site, revealing a complex interaction between the nesting female and the environment. Furthermore, this process shows a high degree of species dependence on the environment. The temperature of the nest selected by the female, not microhabitat, directly determines hatchling sex ratio, suggesting that the evolutionary agent of TSD is nest site selection by females.   Human alteration of the environment introduces a foreign agent into the TSD mechanism that may distort this delicate reproductive and developmental process and produce sex ratios unintended by the females. On a large scale, this may have an inadvertent effect on the terrapin population.

In order to see how nest site selection and TSD influence the overall hatchling sex ratio, I analyzed the sex ratios of all 100 nests I studied. 57% of nests contained nearly single-sex (1 member of the opposite sex in the clutch) and single-sex clutches. Since single-sex clutches are indicative of a species with TSD, this result supports the statistical analyses. Furthermore, there was a higher correlation between dominated nests and the degree to which they were dominated, expressed as the percent of females than there was for males. Since it can be inferred that the female-dominated clutches incubated in nest sites with high percentages of bare ground, the stronger correlation between female nests supports the conclusion that bare ground is an accurate predictor of nest temperature for females.

Identification of relationships between components of the microhabitat, nest temperature, and sex ratio of diamondback terrapins in Jamaica Bay Wildlife Refuge by means of ANOVAs and regression tests, as well as analyses of hatchling sex ratios, led to the conclusion that TSD exists in the field for terrapins and is heavily impacted by nest site microhabitat. The results of the statistical analyses show that nesting terrapins can predict their sex ratios by estimating an average nest temperature based on the amount of bare ground at a nest site. By choosing the temperature in which their offspring incubate, nesting terrapins can control their offspring sex ratio and fitness. Analyses of hatchling sex ratios show that terrapins in Jamaica Bay Wildlife Refuge are choosing to produce more females. Perhaps it is only natural, since females can store sperm and within a single clutch have the offspring of several males. Therefore, small quantity of males in the population is sufficient to maintain genetic diversity. While the nest site selection and the criteria that stand at its foundation are now better understood, the reasons for choosing to produce these sex ratios are still somewhat mysterious. I do not know if it will ever be possible to truly discover what a female terrapin thinks when nesting, but I now know that the discovery will not be made by viewing nest site selection from a human perspective. One year ago I was certain that if terrapins were choosing nest sites on the basis of microhabitat with the purpose of controlling their sex ratio, they were using vegetation as a temperature indicator. How could they not? Hatchlings crawled toward shade. After all, when looking for shade, people look for trees and other vegetation too. From my human perspective, I saw vegetation as a source of shade. I did not see bare ground as the absence of vegetation. I took for granted that I could easily identify shade-providing sources. I only needed to remove my glasses and look around to see that it is not always so easy. Had I been able to talk to a terrapin, she might have been able to point out this fact, now quite obvious, to me. In my case, mathematical analyses

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revealed that which observation, from my biased human perspective, was unable to. The rewards were not only valuable scientific data, but also an invaluable lesson on how great an impact on others and the world one's choices can have. If one day it will be known why terrapins choose to produce the sex ratios they do, I believe the answer will have a mathematical foundation, because just as it is difficult for the human mind visualize a four dimensional world, it is also difficult for the human mind to think like that of another species. Regression tests, ANOVAs, and correlation coefficients were my translators for understanding the terrapin language, as incomprehendable to me prior to my study as the birth and death of stars were to scientists before they knew that $E=mc^2$. In today's highly specialized and sectionalized scientific community, it is easy to overlook the fact that the branches of science are intertwined, and that mathematics and science are inseparable. What distinguishes fact from philosophy is its ability to be expressed mathematically. Thinking in words suffices in our human reference frame, but when we venture to step outside to pursue a probing scientific question, we find that the road which leads to the answer is paved with numbers.

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