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The Role of Nest Depth and Site Choice in Mitigating the Effects of Climate Change on an Oviparous Reptile

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Abstract: Climate change is likely to have strong impacts on oviparous animals with minimal parental care, because nest temperature can impact egg development, sex, and survival, especially in the absence of mitigation via parental care. Nesting females may compensate for increasing temperatures by altering how, when, and where they nest. We examined the factors determining nest depth and site choice as well as the effects that nest depth and location have on nest temperature and hatching success in the diamondback terrapin (*Malaclemys terrapin*). We found that nest depth was not correlated with nesting female size, egg characteristics, or daily temperatures. Nest temperatures and hatching success were correlated with different environmental and nest characteristics between 2004, a cool and wet year, and 2005, a hot and dry year. Females selected nests with lower southern overstory vegetation in 2005. These results suggest that nest depth and location can play an important yet varying role in determining nest temperature and hatching success in more extreme warm and dry environmental conditions and, therefore, may mitigate the impacts of climate change on oviparous reptiles. However, we found minimal evidence that turtles choose nest locations and depths that maximize offspring survival based on short-term environmental cues.

Keywords: diamondback terrapin; hatching success; reproductive fitness; temperature; turtles; behavior

1. Introduction

Global air and ocean temperatures have increased over the past century and current trends are expected to continue and accelerate [1]. Climate change is expected to have widespread impacts on the fitness of aquatic, marine, and terrestrial species throughout the world according to Blaustein et al. [2], Pankhurst and Munday [3], and Grazer and Martin [4]. Organisms may alter their behavior to mitigate the effects of climate change on survival and reproductive success. Warming air and ocean temperatures have already caused poleward and altitudinal range shifts in numerous plant and animal species (reviewed by Walther et al. [5]). Climate change has also affected phenological patterns of many plants and animals, including migration [6–9], flowering [10], and reproduction [11–15].

Oviparous, or egg-laying, animals are particularly vulnerable to climate change because egg development and survival are strongly influenced by ambient temperatures in the nest. This is especially true for species with minimal parental care because such care would provide opportunities for parents to influence nest temperatures after oviposition. Nest temperature has wide-ranging impacts on hatching success, incubation time, and hatchling growth rates [16–21] as well as indirect effects on hatchling size and hatchling activity levels in turtles, lizards, and snakes [16–23]. These effects of nest

temperature on hatchling traits can last up to a year after individuals hatch [21]. Extreme temperatures can lower hatching success [18], and temperature fluctuations also affect incubation time, hatchling activity levels, and hatching success [19,24,25]. Oviparous species with temperature-dependent sex determination (TSD), where offspring sex is determined by the temperature at which embryos develop [26], have additional challenges because changing ambient temperatures can alter sex ratios within a population [27–29]. Average incubation temperature increases as small as 1–2 °C during egg development can result in highly skewed sex ratios in fishes and turtles [14,27,28]. Wide temperature fluctuations during development can also alter sex ratios [25].

Oviparous animals may compensate for changing environmental conditions before incubation by altering where and how they build their nests. Reptiles, including turtles and lizards, choose nest sites that affect the environmental conditions in which their eggs develop. Reptiles select nest sites based directly on temperature [19,30,31] and based on vegetation cover [32–35], which in turn affects mean, minimum, and maximum nest temperatures as well as daily nest temperature fluctuations [29,31,35–38]. Consequently, the degree of shading and vegetation cover can affect offspring sex ratios and incubation time [39–41]. In numerous reptile species, females consistently pick nest sites with the same microhabitat conditions year to year [22,33,42] and pick nest sites that compensate for local temperatures and climate [43,44]. Both striped mud turtle (*Kinosternon baurii*) and painted turtle (*Chrysemys picta*) hatching survival is higher when eggs develop at nest sites selected by females compared to random sites [45,46]. Because nesting females generally select nests sites with environmental conditions that increase offspring survival and repeatedly pick the same type of nest site or pick based on external environmental conditions, female nest site selection may be an adaptation to maximize reproductive success.

Oviparous reptiles may also be able to increase their reproductive fitness by altering the depth at which they lay their eggs [47]. Some reptile species, such as the Highlands Forest-skink (*Anepischetosia maccoyi*), lay their eggs on the substrate surface [22], while the monitor lizard *Varanus panoptes* deposits its eggs as deep as 3.6 m, such as in [48]. The effect of nest depth on nest characteristics is not well understood. Research suggests that nest depth may be correlated with mean nest temperature [47,49–51], temperature fluctuations [41], range of temperatures [48], and maximum temperature [52]. Both exceptionally shallow and deep nests can experience temperature stress that can prevent egg development [49]. Nest depth may be correlated with other development factors, such as incubation time [47,49,53] and offspring sex ratios [47,51]. Nest depth may also be correlated with environmental characteristics within the nest, such as moisture levels [48]. Conflicting evidence suggests that nest depth may [47,54] or may not [55,56] affect hatching success, defined as the percentage of eggs that incubate successfully and hatch. However, because extreme temperatures lower hatching success [18] and nest depth may impact nest temperature maximums, minimums, and fluctuation magnitudes [41,48,49,52], nest depth may impact offspring survival by mitigating extreme daily temperatures or temperature fluctuations.

If oviparous animals can control the depth at which they oviposit their eggs, they could reduce the impacts of warming ambient temperatures on egg development and survival by burying eggs deeper. However, there is conflicting evidence about which factors determine nest depth and whether these animals can control the depth at which they oviposit. Burger [53] found that nest depth was correlated with female carapace length, carapace width, plastron length, egg width, and egg length in diamondback terrapins, and, similarly, nest depth was positively correlated with female size and clutch size in the Amazonian giant river turtle (*Podocnemis expansa*) [51]. Iverson et al. [57] found that nest depth was correlated with female carapace length but not clutch size, clutch mass, or egg size in snapping turtles. Nagle et al. [58] found that map turtle (*Graptemys geographica*) clutch size was positively correlated with nest depth; however, Montevecchi and Burger [59] showed that the diamondback terrapin female size, as measured by plastron length, was not correlated with nest depth. If female size is not correlated with nest depth, meaning females can alter their nests' depths rather

than females being limited in how deep they can build their nest based on their size, varying nest depth may allow oviparous individuals to compensate for climactic changes [31,47,60].

Numerous researchers have suggested that anthropogenic climate change may have a relatively large and negative effect on turtles [61,62]. Post-oviposition parental care is rare among turtles, hence there is little opportunity for them to influence incubation temperatures after oviposition. The diamondback terrapin is an example of a turtle species whose reproductive success is likely to be impacted by climate change. Diamondback terrapins range from Cape Cod, Massachusetts, to Corpus Christi Bay, Texas [63], which encompasses nearly 70,000 km of United States Atlantic coastline, 2000 km north–south, spanning 16.5° latitude and 27.4° longitude and four Koppen climatic zones, plus Bermuda. Diamondback terrapin reproductive ecology across much of this range is well studied [64]. Females nest April–August, although the timing and length of the nesting season varies by location [53,65,66]. Females nest preferentially in sandy and sunny areas with little vegetation [31,66]. Clutch size, egg weight, and nest depth vary extensively both within and between nesting sites [31,53,59,65]. Like most emydid turtles, diamondback terrapins exhibit TSD with a pivotal temperature (=the temperature at which an equal ratio of males and females develop) of approximately 28–29 °C, which varies among sites [67]. Because terrapins are oviparous, have TSD, and have no post-oviposition parental care, temperature changes caused by climate change could affect offspring development, survival, and sex.

We investigated diamondback terrapin nest site choice, nest depth, and the role of both in determining nest temperature and hatching success. Specifically, we sought to determine which factors affect nest depth, whether diamondback terrapins preferentially nest in areas with specific microhabitat characteristics, and how nest depth and site choice affect nest temperature and hatching success. We hypothesized that environmental conditions, rather than the nesting female or egg clutch's physical conditions, would determine nest depth, and that diamondback terrapins would build deeper nests on warmer days to protect nests from extreme temperatures. We also predicted that diamondback terrapins would build nests in areas with less vegetation and higher solar radiation and that these nests would have higher temperatures and hatching success.

2. Materials and Methods

In June and July of 2004 and 2005, we observed female diamondback terrapins nesting on the island of Rulers Bar Hassock in the Jamaica Bay Wildlife Refuge (JBWR; 73°49' W, 40°37' N) in Queens, NY, USA. We allowed females to nest naturally. Once each female started to leave the nest site, she was captured for processing, during which we measured carapace and plastron length, noted any abnormalities, gave un-tagged individuals a unique passive integrated transponder (PIT) tag into the coelom in front of their right hind limb, and scute-notched individuals if not previously marked. We then released females back into the water nearby.

On the day of oviposition, we made five measurements at the nest site to quantify nest microhabitat characteristics. We visually estimated the percent bare ground, percent grasses, percent dicotyledonous plants, and percent leaf litter in the 1 m² area around the nest. There were often multiple layers of plants and leaves, so these percentages sometimes summed to greater than 100%. We differentiated between monocotyledonous and dicotyledonous plants because of their differences in growth form; dicotyledonous plants were generally taller and therefore were more likely to affect overhead cover. We also measured percent overhead cover directly over the nest in each of the cardinal directions using a spherical densiometer, as in Janzen [40]. These measurements were repeated at four randomly chosen locations around each nest. These sites were selected by randomly choosing a direction (north, south east, or west) using a spinner and a distance between 0.5 and 6.0 m using a random number generator.

We then excavated all nests on the day of oviposition and counted and weighed the eggs in grams. Because eggs were moved on the day of oviposition, we assumed the movement would have no detrimental effect on the embryos [68]. We measured nest depth from the ground surface level to the bottommost point of the egg chamber in centimeters. Eggs were placed at their original depth with an Ibutton[®] temperature logger on top. We then re-filled nests with the original soil. Temperature

loggers (DS1922L-F5# Thermochron IButtons, software corrected accuracy to ± 0.5 °C) recorded the temperature of the nest every hour with 0.5 °C accuracy. We recovered the temperature loggers once the hatchlings emerged.

We protected nests from egg predators with predator excluders. In 2004, we protected nests using a square flat piece of 1.27 cm hardware cloth measuring 61 by 61 cm held to the ground with metal stakes and buried using local substrate. Sixty days after oviposition, we removed the hardware cloth and placed a 40.6 by 40.6 cm predator excluder, which was 5.08 cm high, on top of the nest. We also staked this predator excluder into the ground using metal stakes. In 2005, we protected nests with a circular cage made with 1.27 cm hardware cloth measuring 60 cm deep with a diameter of 45 cm. We placed these predator excluders 10 cm into the ground around the nest and staked them into the ground. Subsequent experiments showed that such excluders do not affect nest temperatures [69].

We monitored nests daily until hatchlings emerged. If no hatchlings emerged after 105 days, we excavated the nest. Nests were also excavated after all the hatchlings had emerged or ten days after the last hatchling had emerged. We counted all unhatched eggs, live and dead hatchlings, and root-infiltrated eggs. We measured emerged and excavated hatchlings' carapace and plastron lengths. This work was conducted under Hofstra University IACUC protocol 03/04-9.

We acquired temperature and precipitation records for June, July, and August from 1984–2005 from the John F. Kennedy (JFK) International Airport weather station, which is approximately 5 km northeast of the JBWR. We compared records from 2004 and 2005 to relevant records from the previous 20 years.

Statistical Analysis—Due to the limited storage capacity of the temperature loggers, we were unable to record hourly temperatures for the entire incubation period of some nests. We therefore focused on the temperatures from day 28–35 as a proxy for the entire incubation period. This time period has been shown to be the temperature-sensitive period (TSP) during which sex is determined in diamondback terrapins [67].

We calculated the pivotal-temperature units (PTU) during the TSP using a technique modified from Valenzuela [51]; we used the number of hours that each temperature record was above the pivotal temperature for this population (28.16 °C) [67] multiplied by how much each record exceeded that temperature. For each record that exceeded the pivotal temperature, we subtracted 28.16 °C from the temperature record and added the resulting values. We also calculated the average temperature during the TSP for each nest by averaging the hourly temperature readings during the TSP.

We ran a Welch two sample *t*-test to compare nest depths in 2004 and 2005. We ran simple linear regressions to determine whether female carapace length, clutch size, average egg weight, or average temperature on the day that the nests were oviposited was correlated with nest depth. We examined the residual plots for evidence of non-normality, found none, and proceeded with parametric tests.

We used conditional logistic regression with forward entry ($p \leq 0.05$) for each year's data and both years combined to compare microhabitat data (percent bare ground, percent grasses, percent dicotyledonous plants, percent leaf litter percent, and overhead cover to the north, south, east, west) collected at each nest site to corresponding data collected at the associated random sites. The dependent variable was site type (nest vs. random).

We ran Welch's two-sample *t*-tests and Mann–Whitney tests to compare average temperatures, PTUs, average maximum temperature, average minimum temperature, and average temperature range during the TSP in 2004 and 2005. Then, we ran bidirectional stepwise multiple linear regressions to determine whether these temperature measurements were correlated with nest depth and microhabitat characteristics, including all four physical and five densiometer measurements, each year. We then ran multiple linear regressions using the selected variables.

Hatching success was defined as the proportion of oviposited eggs that resulted in hatchlings that emerged from the nest or were found alive when nests were excavated. An arc-sin square root transformation was applied to the hatching success data from both years to compensate for

non-homogeneous variance. A Mann–Whitney test was used to compare hatching success in 2004 and 2005.

We then ran bidirectional stepwise multiple regression models to determine the significance of all four microhabitat variables, all five densiometer readings (north, south, east, west, and total), nest depth, average nest temperature during the TSP, and PTUs during the TSP on hatching success. We then proceeded with linear or multiple linear regressions using the selected variables.

3. Results

We observed 140 terrapin nests in 2004 and 135 nests in 2005. Means and standard deviations for nest depth, female carapace length, clutch size, and egg weight for both years are given in Table 1. Nest depths were not significantly different in 2004 compared to 2005 ($t = 1.11$, $p = 0.27$). Nest depth was not correlated with female carapace length (2004: $F = 6.9 \times 10^{-7}$, $df = 1.138$, $p = 0.99$, $r^2 = 4.98 \times 10^{-7}$; 2005: $F = 0.20$, $df = 1.133$, $p = 0.65$, $r^2 = 0.0015$), clutch size (2004: $F = 1.1$, $df = 1.138$, $p = 0.29$, $r^2 = 0.0080$; 2005: $F = 0.54$, $df = 1.133$, $p = 0.46$, $r^2 = 0.0041$), or average egg weight (2004: $F = 0.26$, $df = 1.138$, $p = 0.61$, $r^2 = 0.0019$; 2005: $F = 3.0$, $df = 1.133$, $p = 0.086$, $r^2 = 0.022$). Nest depth was also not correlated with average air temperature on the day nests were oviposited in 2004 ($F = 0.76$, $df = 1.118$, $p = 0.38$, $r^2 = 0.0064$) or 2005 ($F = 2.6$, $df = 1.136$, $p = 0.11$, $r^2 = 0.018$).

Table 1. Means and standard deviations (SD) for diamondback terrapin (*Malaclemys terrapin*) female and nest characteristics collected at Jamaica Bay Wildlife Refuge, NY in 2004 and 2005.

Characteristic	2004 Mean (SD)	2005 Mean (SD)
Nest depth	15.2 (1.9) cm	14.9 (2.2) cm
Female carapace length	20.2 (1.4) cm	19.6 (0.9) cm
Clutch size	13.3 (2.6) eggs	12.9 (2.9) eggs
Egg weight	8.7 (0.9) g	8.7 (1.1) g

Analysis of data from both years combined revealed that densiometer readings to the south were significantly different between actual nests and random sites ($B = -0.084$, $Wald = 6.9$, $p = 0.0080$). All other variables failed entry into the model ($p > 0.146$). Analysis of data from 2004 alone revealed that all variables failed entry into the model ($p > 0.185$). Analysis of data from 2005 alone revealed that densiometer readings to the south were significantly different between actual nests and random sites ($B = -0.097$, $Wald = 3.9$, $p = 0.049$). All other variables failed entry into the model ($p > 0.11$).

We collected temperature measurements on 66 nests in 2004 and 118 nests in 2005. Average temperatures, PTUs, maximum temperatures, and minimum temperatures during the TSP were significantly higher in 2005 than in 2004 ($p < 0.05$; Table 2). The temperature range during the TSP was significantly higher in 2004 than in 2005 ($p < 0.05$; Table 2). The bidirectional stepwise multiple regressions showed that densiometer readings to the south, percent bare ground, and nest depth were correlated with average nest temperature, PTUs, maximum nest temperatures, and nest temperature range in 2004. All four of these temperature metrics were negatively correlated with densiometer readings to the south and nest depth and positively correlated with the percent of bare ground ($p < 0.05$; Table 3). Minimum nest temperatures were not correlated with nest depth nor any microhabitat variable in 2004.

Table 2. Results from statistical tests comparing nest temperature measurements during the temperature-sensitive period for diamondback terrapins (*Malaclemys terrapin*) in a particularly cool, wet year (2004) and a hot, dry year (2005).

Variable	2004 Mean (SD)	2005 Mean (SD)	Test Type	Test Statistic	p-Value
Average Temperature	26.1 (1.5) °C	28.1 (2.0) °C	Mann-Whitney	W = 1596	3.3×10^{-11}
Pivotal Temperature Units (PTUs)	149.8 (111.6)	281.9 (182.7)	Mann-Whitney	W = 2194	9.4×10^{-7}
Maximum Temperature	30.6 (2.3) °C	32.4 (2.6) °C	Welch Two-Sample t	T = -4.6	8.7×10^{-6}
Minimum Temperature	22.6 (0.9) °C	25.0 (1.7) °C	Mann-Whitney	W = 1014	$<2.2 \times 10^{-16}$
Range of Temperatures	8.1 (1.9) °C	7.4 (2.0) °C	Mann-Whitney	W = 4616	0.021

Table 3. Results from bidirectional stepwise multiple linear regression model selection and subsequent multiple linear regressions to determine which nest microhabitat and depth variables correlated with nest temperature measurements during the temperature-sensitive period in diamondback terrapins (*Malaclemys terrapin*).

Year	Response Variable	Predictor Variables (Correlation)	F-Statistic	df	R ²	p-Value
2004	Average Nest Temperature	Den S (-) Bare ground (+) Nest depth (-)	3.55	3.62	0.15	0.019
	Pivotal Temperature Units (PTUs)	Den S (-) Bare ground (+) Nest depth (-)	5.28	3.62	0.20	0.0026
	Maximum Nest Temperature	Den S (-) Bare ground (+) Nest depth (-)	6.10	3.62	0.23	0.0011
	Temperature Range	Den S (-) Bare ground (+) Nest depth (-)	8.98	3.62	0.30	5.0×10^{-5}
2005	Average Nest Temperature	Den N (+) Den S (+) Den E (-) Nest depth (-)	6.80	4.112	0.20	6.2×10^{-5}
	Pivotal Temperature Units (PTUs)	Den N (+) Den S (+) Den E (-) Depth (-)	11.04	4.112	0.26	1.4×10^{-7}
	Maximum Nest Temperature	Den N (+) Den S (+) Den E (-) Depth (-)	12.26	4.110	0.31	2.8×10^{-8}
	Minimum Nest Temperature	Den N (+) Den W (+) Bare ground (+) Den E (-)	3.99	3.111	0.097	0.0097
	Temperature Range	Bare Ground (-) Litter (-) Dicots (-) Nest Depth (-)	14.83	5.109	0.40	4.3×10^{-11}

According to the bidirectional stepwise multiple linear regression models, correlations between nest depth, microhabitat characteristics, and temperature metrics were much more complex in 2005. All temperature metrics except minimum nest temperature were negatively correlated with nest depth. Average nest temperature, PTUs, and maximum nest temperature during the TSP were also negatively correlated with densiometer readings to the east and positively correlated with densiometer readings to the north and south. The range of temperatures during the TSP was also negatively correlated with densiometer readings to the east and the percent of bare ground, leaf litter, and dicotyledonous plants. Minimum nest temperatures during the TSP were positively correlated with densiometer readings to the north and west and the percent of bare ground. Table 2 provides an overview of the

variables selected by the stepwise multiple regression and results from the resulting multiple linear regression models.

There was a significant difference between hatching success in 2004 (mean = 77.8%, SD = 29.6%) and 2005 (mean = 63.3%, SD = 33.2%, $W = 11,882$, $p = 0.00040$). According to the bidirectional stepwise multiple regression models, hatching success was affected only by the percentage of bare ground in 2004. Hatching success was positively correlated with the percentage of bare ground in 2004, although the percentage of bare ground explained little of the variation in hatching success ($F = 4.37$, $df = 1.64$, $r^2 = 0.064$, $p = 0.040$). In 2005, however, hatching success was affected by densiometer readings to the east and west, nest depth, average nest temperature during the TSP, and PTUs during the TSP. Hatching success was positively correlated with densiometer readings to the east, nest depth, and average nest temperature but negatively correlated with densiometer readings to the west and PTUs in 2005 ($F = 3.22$, $df = 5.111$, $r^2 = 0.13$, $p = 0.0094$).

In comparison to the climate records from the JFK weather station from 1984 to 2005, 2004 was wet and cool, while 2005 was dry and hot. July–August of 2004 was in the upper 10th percentile for total precipitation and lower 25th percentile for average temperature compared to that same period in 1984–2003. July–August 2005 was in the lower 15th percentile for total precipitation and the upper 10th percentile for average temperature.

4. Discussion

By selecting nest site characteristics and altering cavity characteristics, such as nest depth, turtles and other oviparous species may be able to mitigate the impacts of climate change on reproductive success. We investigated the factors determining nest depth, whether females preferentially select nest sites with particular microhabitat characteristics, and the effect of nest depth and site choice on nest temperature and hatching success in diamondback terrapins. Our results show that nest depth is an important determinant of nest temperature during subsequent incubation, although the importance of nest depth may vary yearly. Our results agree with previous research showing that nest depth is negatively related to mean temperatures [47,49–51], maximum temperatures [52], and temperature ranges [41,48]. Understanding the determinants of nest temperature is particularly important for species with TSD, such as diamondback terrapins. Because deeper nests had lower PTUs during the TSP in both years, nest depth likely has an impact on hatchling sex ratios. Climate change is expected to alter sex ratios in oviparous species with TSD [27,28,62], but deeper nests may be less affected by these changing temperatures.

Despite its role in determining nest temperature, nest depth was only related to hatching success in one of the two years. Nest depth and hatching success were positively correlated in 2005, which was an unusually hot and dry season at the JBWR with higher nest temperatures than the previous year. Previous research has found conflicting evidence about the relationship between nest depth and hatching success [47,54–56]. These results build on previous research by demonstrating that this relationship can vary with yearly weather. In years with favorable environmental conditions, hatching success is unrelated to nest depth. As environmental conditions become more unfavorable, nest depth has an increasingly important impact on hatching success, likely because shallower nests experience extreme temperatures and temperature fluctuations that could be detrimental to developmental success. This trend suggests that nest depth could play an important role in moderating the effects of climate change on reproductive success.

A key step in understanding the relationship between nest depth and the effects of climate change on oviparous animals is to determine the factors influencing nest depth. Existing research provides conflicting results about whether nest depth is determined by nesting females and egg physical characteristics, such as female size, egg size, and clutch size [47,51,53,57–59]. We found that female size, clutch size, and average egg weight had no impact on nest depth. If nest depth is not inflexibly determined by physical characteristics, such as a female's size, turtles may be able to alter their nests' depths and thereby lessen the impacts of extreme temperatures and temperature variations caused by

climate change [31,47,60]. However, we also found that nest depth was not correlated with average temperature on the day of oviposition. Average nest depth also did not vary between an unusually hot year and a colder year. These results suggest that either diamondback terrapins do not alter the depth of their nests, or, if they can control the depth of their nests, are not reacting to short-term temperature cues.

Our results demonstrate that diamondback terrapins generally select nest sites with more open canopy in the southern direction, but not based on any other microhabitat characteristics. Contrary to our results, most studies suggest that diamondback terrapins and other turtle species select nest sites based on vegetation proximity, density, and height [31,35,44,45]. In most of these cases, however, local vegetation, such as grasses, was also correlated with nest temperature [31,35]. We did not find a relationship between the density of grasses or dicotyledonous plants and most nest temperature metrics at the JBWR. Because low-lying vegetation does not play an important role in nest temperatures, diamondback terrapins at the JBWR may not select nesting sites based on vegetation, as do other species and populations. Alternatively, other species and populations may select vegetated nesting sites because vegetation affects how easily an individual can dig in the sediment [32] and nest predation rates [70]. These factors do not appear important for diamondback terrapin at the JBWR as they did not select nesting sites based on vegetation.

Various microhabitat characteristics, including overstory vegetation cover to the south, were important determinants of nest temperatures. In the cool, dry year, most nest temperature measurements were correlated with southern overstory vegetation cover and the percent bare ground. In the warmer, wetter year, most nest temperature measurements were affected by not only southern overstory vegetation cover and percent bare ground, but also northern and eastern overstory vegetation cover and the percentage of leaf litter and dicotyledonous plants. Weisrock and Janzen [37] also found that painted turtle nest temperatures depended on overstory vegetation cover, although Morjan [44] found that canopy cover did not affect nest temperatures. The role of northern and eastern exposure as well as leaf litter and dicotyledonous plants in the warmer year, but not the cooler year, suggests that these microhabitat characteristics become increasingly important as ambient temperatures become more unfavorable, similar to the way nest depth becomes more important with increasing air temperatures.

Female diamondback terrapins at the JBWR select nest sites based on a microhabitat characteristic (southern overstory cover) that affects nest temperatures but not hatching success. Choosing nests based on southern overstory vegetation cover may be an adaptation to increase the likelihood that eggs incubate at optimal temperatures for development or to control offspring sex ratios. However, nest depth and site choice did not compensate for changes in environmental conditions over this two-year period. Ultimately, hatching success was lower and most nest temperature measurements were higher in the hot, dry year than in the cool, wet year. These results suggest that diamondback terrapins may not be able to readily compensate for changing temperatures due to climate change by altering nest depth and location.

Although we found little evidence that diamondback terrapins respond to environmental conditions by altering nest characteristics that affect hatching survival, terrapins and other oviparous animals could respond to long-term temperature cues or other environmental conditions when determining nest depth and location. We only studied nests over a two-year period, which may not be long enough to observe population-level changes in response to climate change. Turtles may alter nest construction behavior over a multi-year period to compensate for long-term climatic changes. Turtles may also use a variety of other environmental cues, such as water temperature, soil temperature, and soil moisture, to determine the optimal nest depth. Future research should study how nesting turtles react to long-term environmental cues and how nest depth within a population may change over time.

5. Conclusions

As climate change continues to cause more extreme temperatures and temperature fluctuations, temperature will have an increasingly severe effect on offspring sex ratios and survival in species

with TSD [61,62]. Our results suggest that maternal nest site selection and nest construction can mitigate the effects of climate change by lowering nest temperatures and temperature fluctuations. However, diamondback terrapins do not appear to select nest locations and depths that optimize offspring survival. Despite the strong connection between nest depth and nest temperatures as well as the correlation between nest depth and hatching survival in an unusually hot year, we did not find evidence that turtles altered their nest depth based on environmental conditions. The only microhabitat characteristic that affects nest location—southern overstory vegetation cover—had variable effects on nest temperatures between years and had no effect on hatching survival. It is possible that turtles may be able to adapt over a longer timescale; however, current evidence suggests that they do not build nests in ways that will mitigate the harmful effects of climate change on survival.

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