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Impact of Environmental Factors on Potential Nest Environment of the Painted Turtle (Chrysemys picta)

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(*Chrysemys picta*)

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Abstract

The painted turtle (*Chrysemys picta*) has temperature-dependent sex determination, meaning that the temperature of the nest determines sex. Vegetation cover of nests has been found to affect the hatchling sex ratio, indicating that there is an indirect causal relationship between sex ratio and vegetation cover. This study looked at the relationship between thermal nest environment and environmental variables (nest temperature and soil composition). Simulated turtle nests were created around a lake located on Elizabethtown College's campus in Elizabethtown, Pennsylvania in locations with varying amounts of cover. A temperature data logger was placed in each nest to record the potential incubation temperature from the end of May to mid-July of 2017. Densiometer readings were taken to determine percent cover at each location. Soil composition was also determined for each nest site by using the soil hydrometer method. It was found that as amount of cover increased, average nest temperature decreased significantly. There was no significant relationship between soil composition and nest temperature. These results could help in assessing the impacts of land use changes around turtle ponds.

Introduction

The painted turtle *(Chrysemys picta*) has temperature-dependent sex determination, meaning that the temperature of the nest determines the sex of the young that hatch. Once a female turtle lays its eggs, it covers the nest and leaves it to develop under environmental factors such as sun exposure, soil type, and vegetation (Gutzke et al., 1987). The fate of the nest environment, and the sex of the offspring, is now determined by these factors. This phenomenon has been closely studied in laboratories and it has been found that for painted turtles, eggs incubated at cooler temperatures favor the development of males, while warmer temperatures favor the development of females (Paitz et al., 2010). In natural nests, environmental factors could potentially skew the sex ratios of local populations by favoring one end of the temperature range over the other, creating hatchlings of a majority of one sex for one or more breeding seasons (Schwarzkopf & Brooks, 1987).

Nest site characteristics play an important role in the sex ratios of painted turtle hatchlings (Janzen, 1994). Freshwater turtles lay their eggs on land and thus the nature of the land in which they dig their nests may strongly influence the thermal environment of those nests. Vegetation cover of nests has been found to impact the sex ratio of painted turtle offspring, indicating that there is a causal relationship between sex ratio and vegetation cover (Weisrock & Janzen, 1999). While this important relationship has been noted, little research has been done to discover a link between vegetation cover and nest temperature (Weisrock & Janzen, 1999). Different land use types have varying levels of vegetation cover. It would make sense then that nests laid in different land uses will be incubated at different temperatures, favoring one sex of hatchlings over the other.

While the painted turtle is classified as a species of least concern by the IUCN Red List, insights into their nesting habits and soil temperature could be applied to other ground-nesting species that exhibit temperature-dependent sex determination (Van Dijk, 2011). This is especially important as warming temperatures as a result of climate change could lead to the production of single-sex hatchlings and then eventually to extinction (Hays et al., 2003). Considerable research has been aimed at measuring the nest incubation temperatures and estimating the sex ratio of marine turtle hatchlings, but little research has focused on freshwater turtle species (Hays et al., 2003). Janzen's research on temperature-dependent sex determination in freshwater turtles focused on air temperature instead of soil temperature (Janzen, 1994). Collecting such information will be useful in assessing the future impacts of climate change and any associated global temperature increases on terrestrial populations. It is hypothesized that thermal environment of simulated nests will vary by amount of canopy cover. Nests created in more exposed environments, like lawns, will experience higher temperatures than nests created in more closed-canopy environments like meadows and forests.

Materials and Methods

Study Area

This study was conducted on Elizabethtown College's campus which is located in Elizabethtown, Pennsylvania. The campus contained three standing, persistent bodies of water in which painted turtles were regularly observed. The pond called Weird Pond was selected for this study because it had three different land use types surrounding it: lawn, forest, and meadow. These land use types were chosen for their variation in canopy cover, ranging from zero cover in lawn to completely covered in the forest.

Simulated Nests

Simulated turtle nests were constructed around Weird Pond. The locations of the nests around the body of water were randomly generated (**Figure 1**). Each nest was placed within 150m of the water's edge and placed in one of three habitat types: lawn, meadow, or forest. Nests were created by digging a 10cm deep hole that was roughly 5cm wide. A temperature data logger (HOBO Pendant 8K) was buried in each nest and a wire cage was placed around each nest to avoid disturbance by animals. The data loggers were placed at the end of May 2017 and were removed on July 27, 2017. Simulated nest temperatures were recorded every hour for the duration of the experiment. Upon removal, a soil sample was taken from each nest location and densitometer readings were taken so that percent cover could be calculated. Soil composition was determined using the soil hydrometer method.

Statistical Methods

Data was analyzed using a multiple linear regression with land cover and soil composition as independent variables and daily mean temperature, daily maximum temperature, daily minimum temperature, and daily range as dependent variables.

Results

Originally, twenty simulated nests were created each with their own data logger. Upon logger removal, five nests had been disturbed and these were not used as part of the results. Of the fifteen nests analyzed, seven were considered open (percent cover <50%) and eight were considered closed (percent cover >50%). The mean daily temperature averages, maximums, and minimums can be seen in **Table 1**. In each case, the daily mean variable for open nests was higher than that of the closed nests.

Of the 15 nests, nine experienced mean daily temperatures above 21.5°C, the minimum temperature at which painted turtle eggs have been shown to develop (**Figure 2**). Only one nest experienced temperatures averaging about 27.0°C, which is the transition zone for painted turtles. More open nests were shown to have greater temperature fluctuations than closed nests, which had more constant conditions (**Figures 3 & 4**).

The soil that nests were placed in was composed mostly of sand, with an intermediate amount of silt, and the least amount of clay. Of sand, silt, and clay, clay individually had the most significant impact on nest temperature with a p-value of 0.045. The individual effects of sand, silt, and clay on nest temperature can be seen in **Figures 5, 6, and 7**. When soil temperature and cover together were analyzed for impact on nest temperature, soil composition was no longer significant. Amount of cover is the variable that has the most significant impact on simulated nest temperature.

Amount of cover was the variable with the strongest impact on mean daily nest temperature. Simulated nest mean daily temperature varied significantly with amount of cover, with a p-value of <0.001. Nest temperature was negatively correlated with amount of cover. Nests with higher percentages of cover tended to have lower mean daily temperatures while nests with less cover tended to have higher mean daily temperatures (**Figure 2**). Maximum daily nest temperature, minimum daily nest temperature, and daily temperature range were also all negatively correlated with amount of cover (**Figures 8, 9, & 10**). All relationships were statistically significant, with all p-values found to be <0.001. Consequently, the most influential factor on thermal nest environment appears to be amount of canopy cover, with soil composition having minimal effects.

Tables and Figures

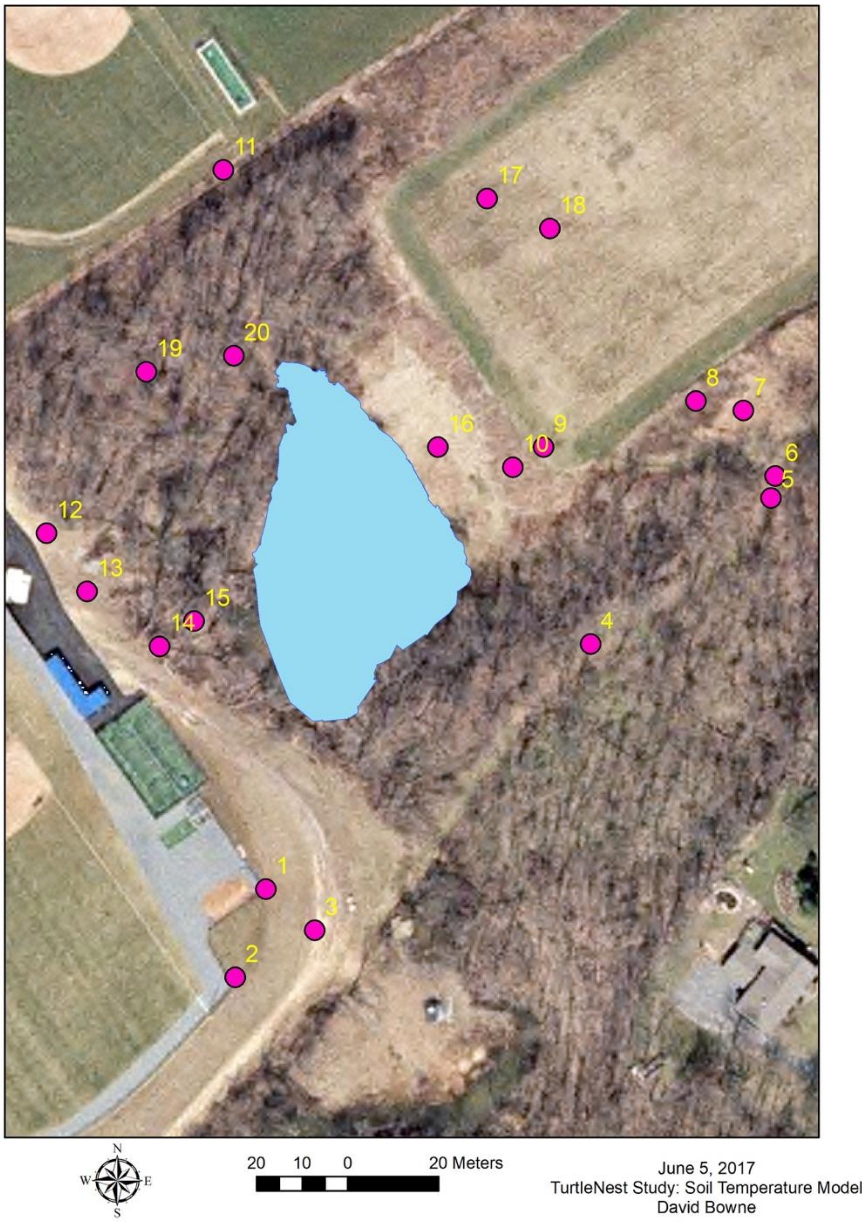

Figure 1. Map depicting the randomly generated locations around Weird pond at which simulated nests were created.

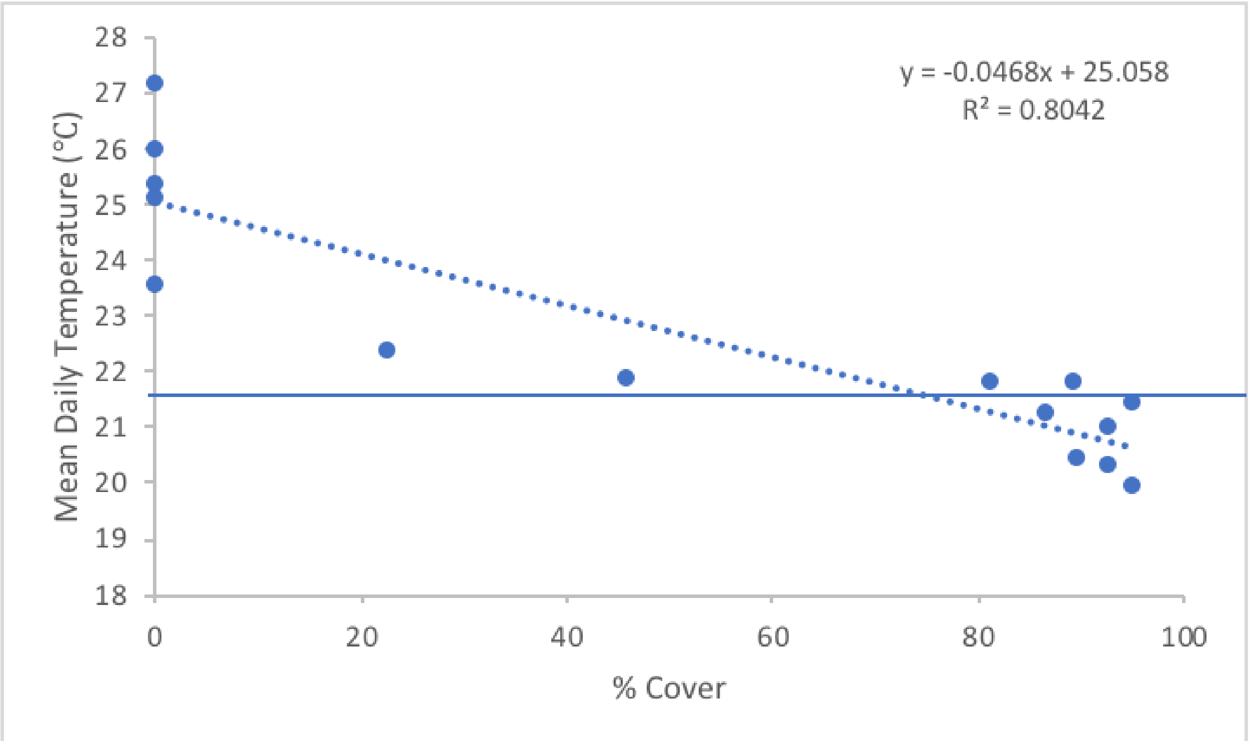

Figure 2. Mean daily temperature plotted as a function of percent canopy cover. The line across the graph is at 21.5°C, which is the minimum incubation temperature required for painted turtle development. There was a significant statistical relationship found between cover and mean daily temperature, with a p-value <0.001.

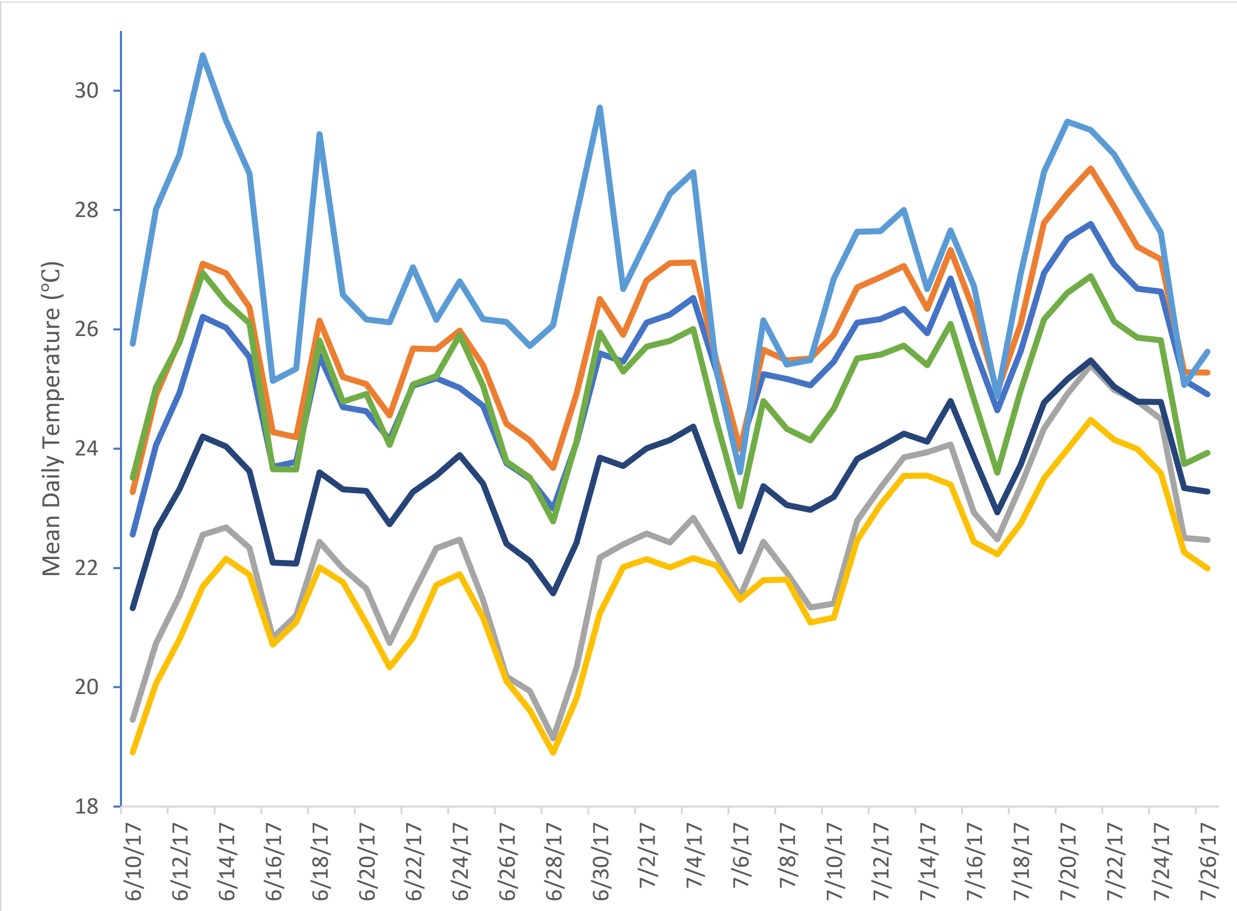

Figure 3. The mean daily temperatures from June 10, 2017 – July 26, 2017 for the 7 loggers that were incubated in an open environment.

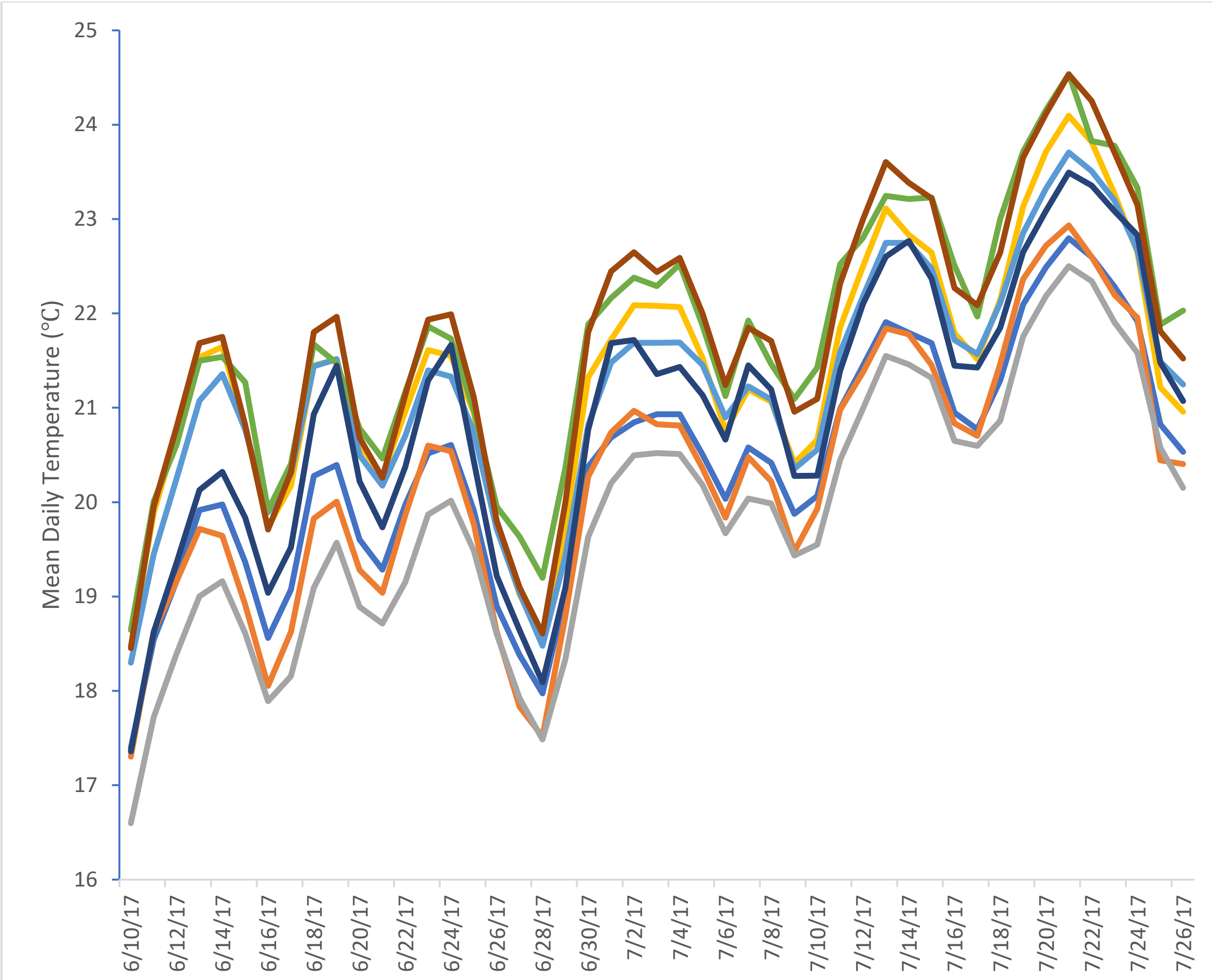

Figure 4. The mean daily temperatures from June 10, 2017 – July 26, 2017 for the 8 loggers that were incubated in a closed environment.

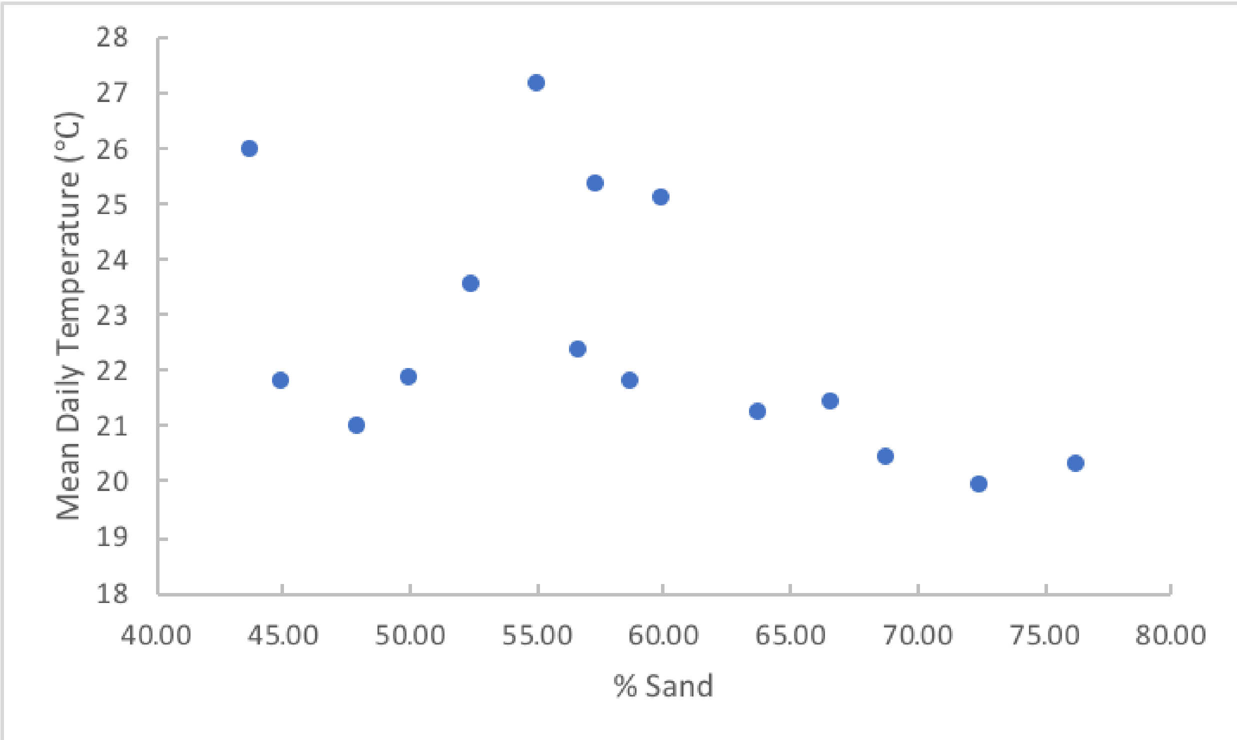

Figure 5. Mean daily temperature plotted as a function of percent sand present in the soil. There was no significant statistical relationship found, $p = 0.089$.

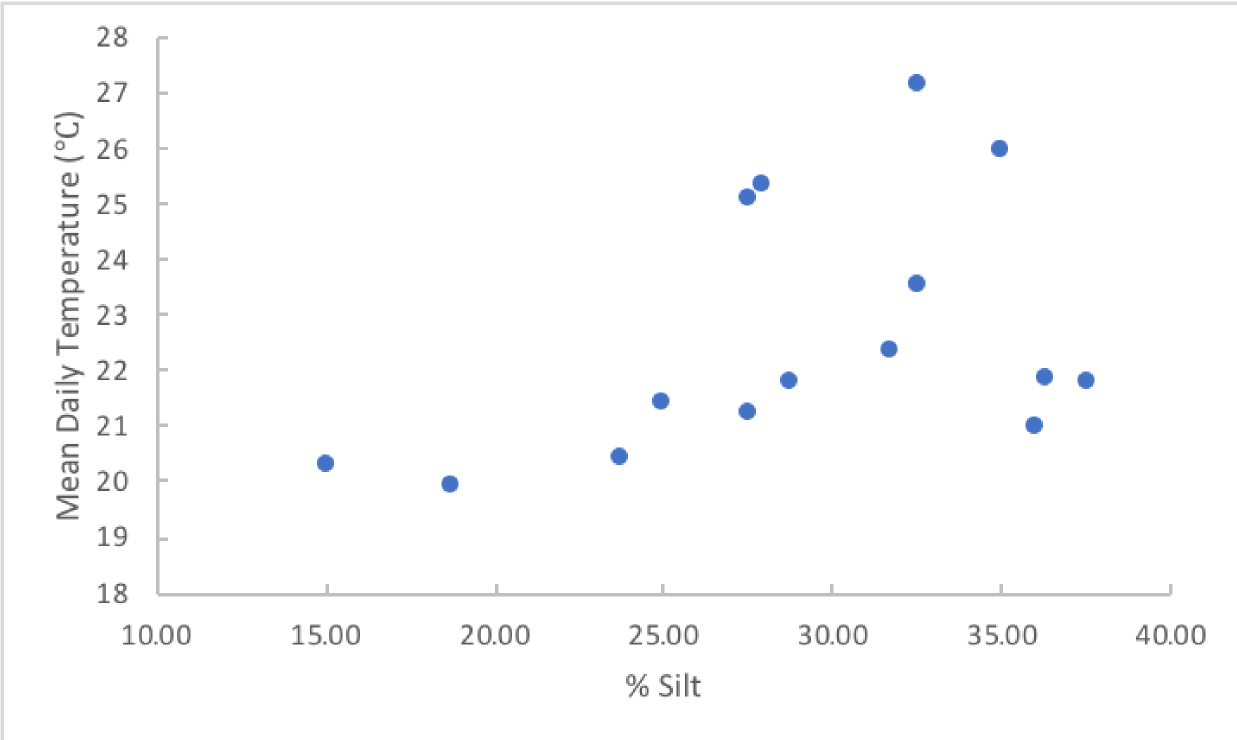

Figure 6. Mean daily temperature plotted as a function of percent silt present in the soil. There was no significant statistical relationship found, $p = 0.160$.

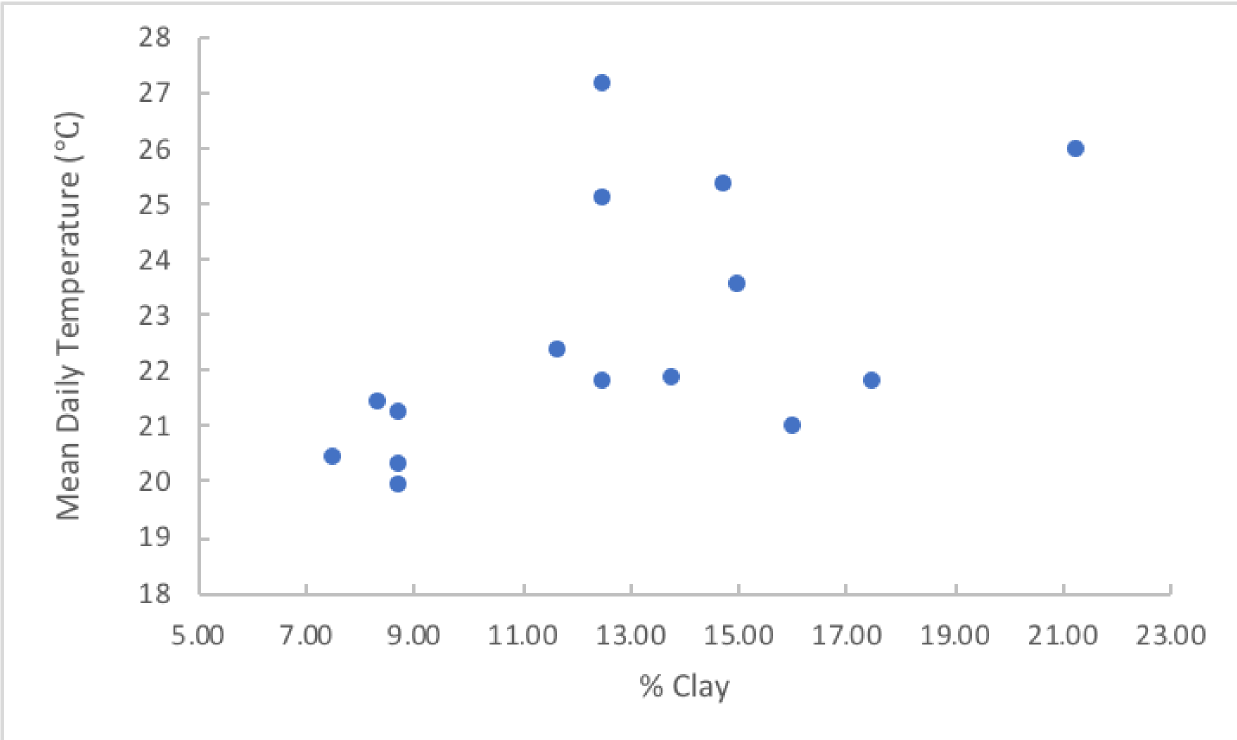

Figure 7. Mean daily temperature plotted as a function of percent clay present in the soil. There was a significant statistical relationship found, $p = 0.045$.

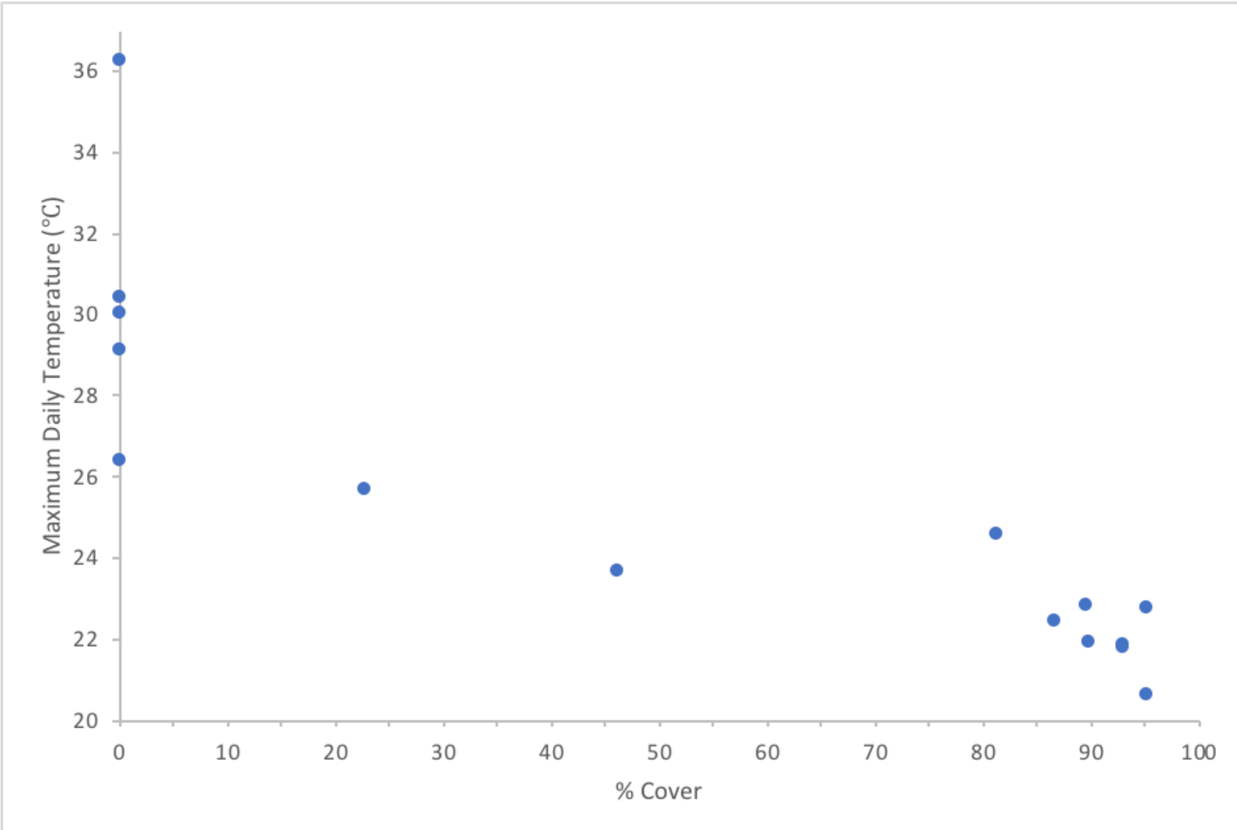

Figure 8. Maximum daily nest temperature plotted as a function of percent canopy cover. There was a significant statistical relationship found between cover and maximum daily temperature, with a p-value <0.001.

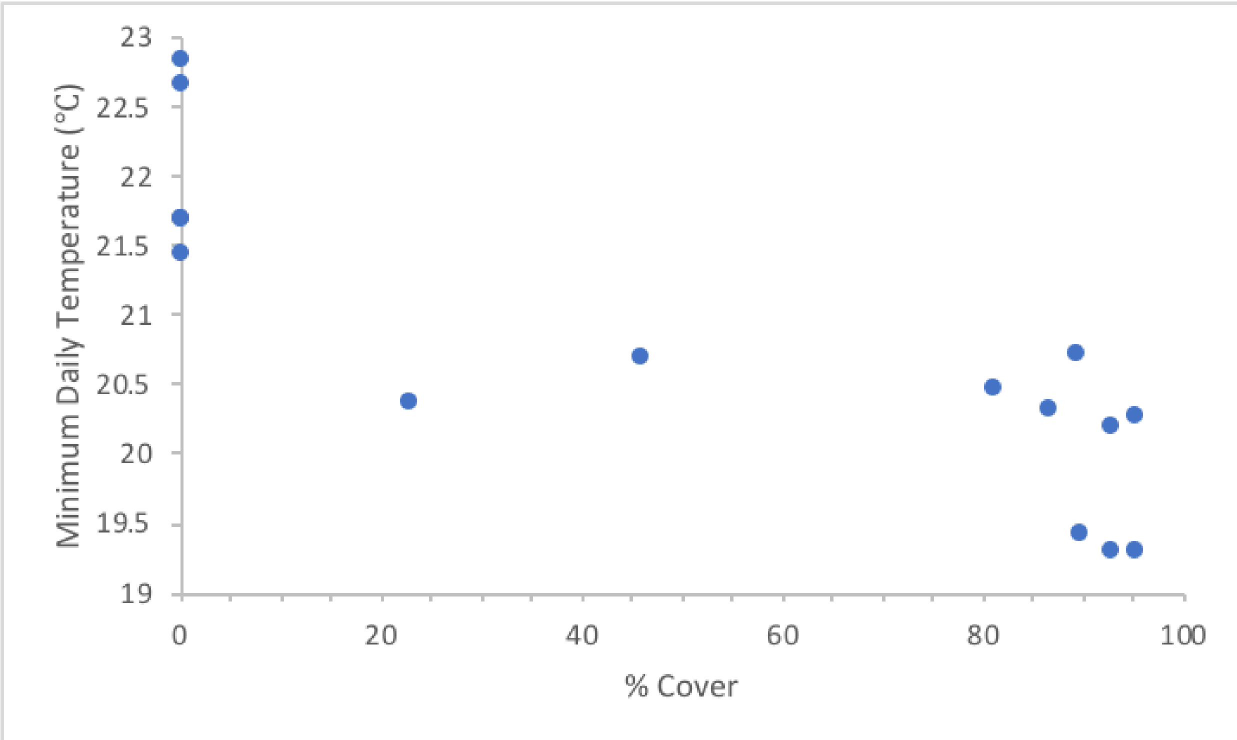

Figure 9. Minimum daily nest temperature plotted as a function of percent canopy cover. There was a significant statistical relationship found between cover and minimum daily temperature, with a p-value <0.001.

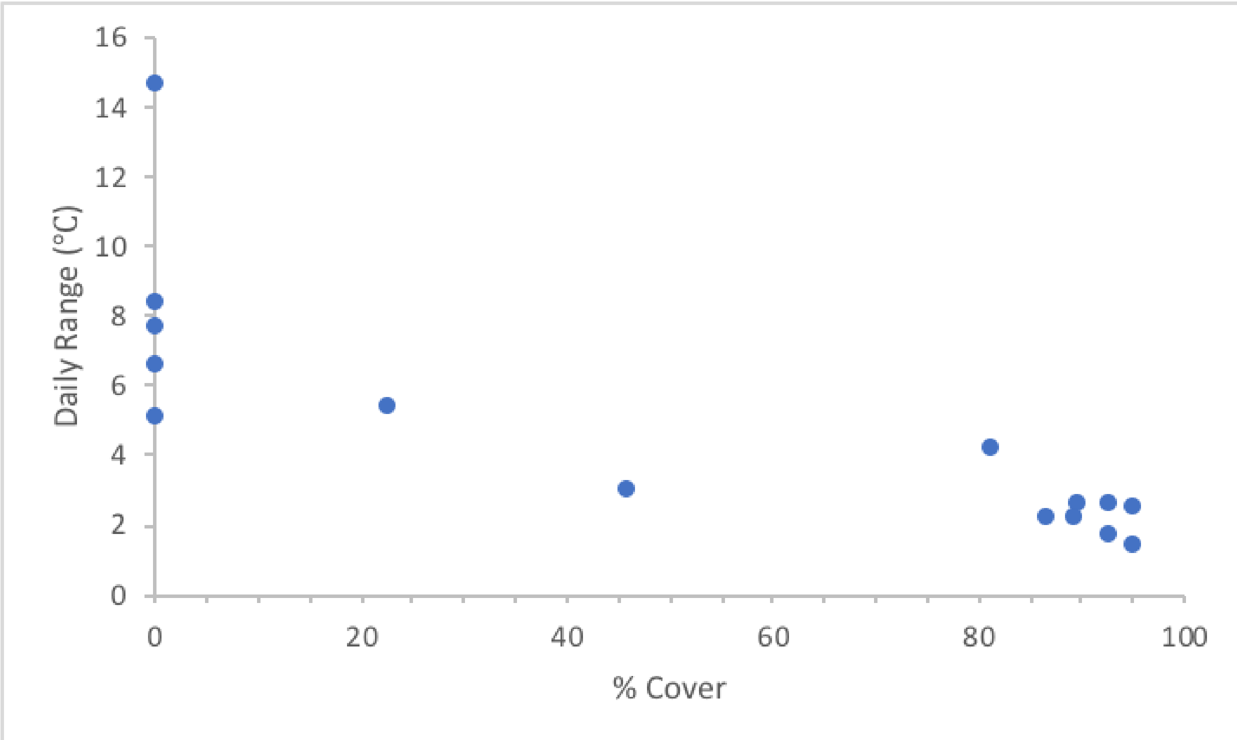

Figure 10. The daily range in temperature plotted as a function of percent canopy cover. The less cover present, the larger the range of temperatures experienced at that site. The more covered the simulated nest site, the less temperature variation experienced. There was a significant statistical relationship found between cover and daily temperature range, with a p-value <0.001.

Table 1. Table of daily mean temperature comparisons for open nests versus closed nests.

	Open Nests	Closed Nests
Average Daily Mean	24.45°C	20.95°C
Maximum Daily Mean	28.80°C	22.35°C
Minimum Daily Mean	21.62°C	19.99°C

Discussion

Statistical analysis showed that overall, thermal nest temperature is primarily influenced by amount of canopy cover. Lower simulated nests temperatures can be explained by greater amounts of canopy cover and higher nest temperatures can be explained by more open nesting environments. Generally, it has been found that higher nest temperatures lead to majority female nests while lower incubation temperatures lead to majority male nests (Ewert & Nelson, 1991). This relationship between canopy cover and temperature agrees with a study conducted by Weisrock and Janzen. They found nest temperature to be negatively correlated with vegetation cover (1999). Another study done by Janzen (1994) also found the same negative correlation between cover and nest temperature with the western painted turtle, *Chrysemys picta bellii*. Janzen found that most nests produced hatchlings of all one sex and nests with more cover tended to produce all male or mostly male hatchlings than more exposed nests.

Even though the nests in this study were simulated, the potential offspring that would have been generated from the nests can be predicted based off the painted turtle transition zone. A transition zone is the incubation temperature at which the majority of eggs incubated above that temperatures will be all one sex while the eggs incubated below that temperature will be the other sex. For painted turtles, the transition zone has been found to be 27.0°C (Paitz et al., 2010). Nests incubated above that temperature yield primarily female offspring, while nests incubated below that temperature are primarily male. Only one nest in my study was incubated at a mean daily temperature above 27.0°C. This suggests that had the nests contained real eggs, only one would have resulted in a majority of female hatchlings. While use of the transition zone serves as a straight forward way to predict hatchling sex ratio, the study used to determine the transition zone was conducted in a laboratory setting.

Many studies conducted on nest incubation temperature were conducted in laboratory settings at one stable temperature (Bull & Vogt, 1979; Weisrock & Janzen, 1999). In natural environments, it is hard to predict what the ratio of male to female offspring resulting from a nest incubated at fluctuating temperatures will be. A paper by Paitz et al. (2010) found that greater temperature fluctuations during incubation tended to result in majority female nests while more constant incubation temperatures resulted in predominantly male nests. More specifically, nests with 4°C of fluctuation from the mean daily temperature resulted in nests that were 30% female while nests with 8°C of fluctuation from the mean resulted in 100% female nests. In natural nest environments, it seems logical that a combination of mean incubation temperature and fluctuations around the mean would best explain hatchling sex ration. While this relationship is interesting, I did not investigate how fluctuations around the mean would influence hatchling ratio.

The amount of cover is the most important factor in predicting simulated nest temperature. The effects of soil composition on thermal nest environment are negligible when compared to the effects of canopy cover. The more open the vegetation cover over a nest was, the higher the mean incubation temperature experienced by the simulated nest. Closed canopy nests experienced lower incubation temperatures. That being said, six of the eight closed canopy nesting sites were incubated below the threshold necessary to produce viable hatchlings (21.5°C). Only one nest out of the nine nests that could have potentially produced viable offspring was incubated above the transition zone of 27.0°C. This means that only one nest would have resulted in a majority of female hatchlings. This indicates that painted turtles' primary sex ratio on Elizabethtown college's campus are likely being skewed towards majority male. If these trends continue into the adult population and over more than one breeding season, it could result have devastating effects on the local turtle population. According to the National Weather Service, the mean air temperature for State College, Pennsylvania in June of 2017 was 22.9°C, while the historic mean was 21.9°C. The mean air temperature for July of 2017 was 25.3°C, while the historic mean was 24.3°C. These air temperatures suggest that the summer of 2017 was not an abnormally cool year, indicating that the lower soil temperatures experienced, which favor male painted turtle development, might be standard.

As areas become more urbanized, turtles will lose closed habitats like forests and meadows and gain exposed habitats like lawns. Turtles do not typically nest in forests, so loss of forest will have little impact on them. Meadows typically have intermediate amounts of cover that are likely to produce lower incubation temperatures with less temperature fluctuations. As more and more turtles are forced to nest in lawn-like, open habitats, their nests will experience higher incubation temperatures and more extreme fluctuations. In order to better understand what this means for painted turtles and for other species with temperature-dependent sex determination, future studies should investigate the combined effects of mean incubation temperature and fluctuations around the mean. Additionally, this study could be re-created using real eggs or nests instead of simulated ones so that the true hatchling sex ratio based on amount of canopy cover could be determined.

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