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HATCHLING SEX RATIOS AND LOCOMOTOR PERFORMANCE OF MIDLAND PAINTED TURTLES (*CHRYSEMYS PICTA MARGINATA*)

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ABSTRACT: The primary objectives of this study were to understand how canopy cover and nest temperatures affect hatchling sex ratios and locomotor performance (i.e., swimming sprint speed and righting response) of *Chrysemys picta marginata* nests. Seventeen nests were monitored with temperature data-loggers during the 2009 nesting season and found to contain 100% male-biased clutches with a mean nest temperature range of 20.0–24.0°C during the thermosensitive period (TSP). The percentage of canopy cover over each nest was inversely and significantly correlated with mean nest temperatures experienced during the TSP. Mean nest temperatures (MNT) did not have a statistical effect on either measure of locomotor performance; however, there was an observed trend toward increased performance with increased MNT.

INTRODUCTION

Turtle species that exhibit temperature-dependent sex determination (TSD) Type Ia may be able to influence the outcome of secondary sex ratios by choosing to nest in areas of more or less shade (Janzen, 1994; Vogt and Bull, 1984; Weisrock and Janzen, 1999). During the middle third of incubation, known as the thermosensitive period (TSP), male and female hatchlings will be produced below and above a pivotal temperature (T_{piv}) (Vogt and Bull, 1982). This study raises the possibility that females may select nest sites based on vegetative cover, and thereby directly influence the hatchling sex ratio. Additionally, gravid females may also alter embryonic survival and locomotor performance of hatchlings by choosing to nest in relatively warmer sites (Ewert et al., 1994; Morjan, 2003; Schwarzkopf and Brooks, 1987; Wilson, 1998).

Temperature plays an integral part in locomotor performance of turtles and likely is a limiting factor that affects their fitness, particularly post-emergence survival as their cartilaginous shells provide little to no protection (Elnitsky and Claussen, 2006; Janzen et al., 2000). Hatchling aquatic turtles disperse from their nest-site to an aquatic habitat, and they require effective locomotion to escape predation and to function as efficient foragers once in the water (Elnitsky and Claussen, 2006; Janzen et al., 2000). Though several studies outline the effects of hydric properties experienced during incubation on the locomotor performance of *Chelydra serpentina* (Finkler, 1999; Miller, 1993; Miller et al., 1987), there are relatively few that focus on temperature (Janzen, 1995), and more specifically on *Chrysemys picta* (Elnitsky and Claussen, 2006; Zani and Claussen, 1994).

In a study by Janzen (1995), *C. serpentina* hatchlings from cooler incubation temperatures swam faster, whereas those from warmer incubation temperatures tended to run more frequently (rather than stay immobile), implying differences in anti-predator behaviors that could be correlated with differences in fitness potential. The righting response of *C. serpentina* has also been shown to correlate directly with environmental temperature, where hatchlings righted faster as temperatures increased (Steyermark and Spotila, 2001). Likewise, locomotor performance measures (burst speed and righting response) of *C. picta bellii* were found to increase with environmental temperature (Elnitsky and Claussen, 2006). Overall, it is thought that population stability is directly linked to greater locomotor performance of individual hatchlings (Steyermark and Spotila, 2001). However, there are many uncertainties on whether these differences in performance are linked to a controllable source (i.e., females influencing these differences through nest site selection).

The primary objectives of this study were to understand how canopy cover affects nest temperatures, and in turn how it affects the hatchling sex ratios and locomotor

performance (i.e., swimming sprint speed and righting response) of *C. p. marginata* nests. We predicted that nests with a greater amount of shading would have lower mean nest temperatures (MNT), and therefore produce male-biased clutches with hatchlings that have faster sprint speeds and righting responses than nests in more sunlit locations.

MATERIALS AND METHODS

Field Design — This study was conducted at The Rookery, Geauga County, Ohio, USA (41.499836°N, 81.292358°W; Geauga Park District, 2008). Forty-nine mid-day visual surveys were conducted in appropriate nesting habitat from 8 May–26 June 2009. Thirty-five nests were located by visual surveys of nesting females; 18 contained eggs that did not hatch, and 17 contained eggs from which live hatchlings were collected. When a nest was located, it was fitted with a 46 X 46 cm wire mesh (0.5 X 0.5 cm) cage to prevent predator disturbance (Doody, 1995; Spetz, 2008). At approximately 10 cm outward from the edge of the cage, an enclosed HOBO TEMP H8 or HOBO U-10 Temperature Data Logger (Onset Computer Corporation) was buried at approximate middle nest depth (5–6 cm) to record the incubation temperatures every 15 minutes throughout the season; these data points were used to calculate MNT during the thermosensitive period (TSP) to analyze the hatchling sex ratio. The TSP was calculated as the middle 25 days of a 75 day incubation period for each nest (J.D. Congdon, pers. com.). Additionally, MNT was calculated across the entire 75 day incubation period to analyze the effects on locomotor performance. Due to the small flask-shaped nest structure of *C. picta* species (Harding, 1997), the data loggers were placed outside of the enclosure to ensure that no disturbance was created to the nest or enclosure when data was being collected throughout the incubation period.

Percent canopy cover and MNT were recorded at each nest. Percent canopy cover (N, E, S, W), which included both vegetation and man-made objects, was measured on 25 July (within or just after the TSP for each nest) using a foresters Model-A spherical densiometer (Janzen, 1994; Janzen and Morjan, 2001; Weisrock and Janzen, 1999). Mean nest temperatures MNT were calculated using HOBOware Lite v2.7.2 and HOBO BoxCar Pro v4.3 (Onset Computer Corporation).

Laboratory Design — Thirty-five *C. p. marginata* nests were monitored and allowed to naturally incubate during this study; however, only 17 nests contained clutches that hatched. After all nests were predicted to complete incubation (75 days; J.D. Congdon, pers. com.), they were excavated 27–30 September 2009 on a sequential basis. Seventy of 97 hatchlings were randomly-selected, and no more than 20 hatchlings were transported per day to ensure that all were subjected to the locomotor performance trials equally and euthanized immediately. Hatchlings were transported in clear plastic containers with substrate from the nests, and the remaining individuals not selected for trials were immediately released at the water's edge upon removal from their nests.

Each hatchling was subjected to a set of 3 swimming sprint speed trials (with a 45–75 min rest between trials) by placing them in a 10 cm X 2 m water track filled with 2.5 cm distilled water maintained at 20.1°C. Not every trial sequence had equal numbers of individuals because the sub-set of nests to be excavated on any given day had either fewer or greater numbers of individuals; this inherently caused variation in resting time between trials. Room temperature was kept at a constant 25°C to replicate nest temperatures typical of hatchling emergence during spring, as it is known that most hatchling *C. picta* in northern latitudes over-winter in the nest (Harding, 1997; Elnitsky and Claussen, 2006; Weisrock and Janzen, 1999). The middle of the track contained a 1 m ruler affixed to the bottom to measure distance sprinted in 60 s. Sprint speed was calculated as distance (cm) / time (s). A score of "0" constituted no response; the 3 trials were averaged for each individual.

Each hatchling was then allowed 45–75 min resting periods before the righting trials were initiated. Each hatchling was subjected to 3 sequential righting trials on a flat laboratory bench with a 15 s rest between flips. During each righting trial, hatchlings were placed individually on their carapace and given a total of 60 s to complete the trial by returning right side-up. A score of "60" constituted no response; all 3 trials were averaged for each individual.

All hatchlings were euthanized under standard humane protocol with an overdose injection of a 1:1 Nembutal solution (Na$^+$ pentobarbital; Janzen, 1994). All specimens were fixed in 10% buffered formalin and preserved in 70% ethanol (Janzen, 1994). Justifications for sacri-
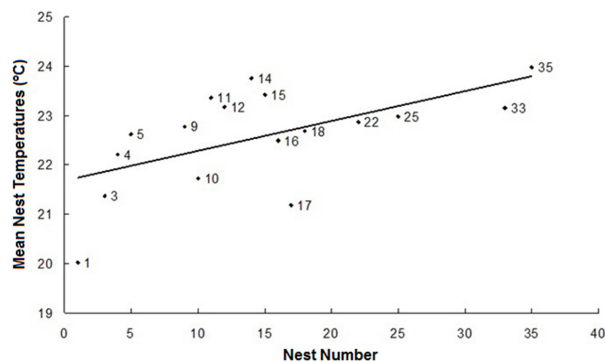
FIGURE 1. Scatterplot of mean nest temperatures (MNT) of 17 *C. p. marginata* nests at The Rookery (Geauga County, Ohio). Temperatures were recorded during the thermosensitive period (TSP), and exhibited temperatures well below the pivotal temperature (T_{piv}) of 28.5°C, suggesting 100% male-biased hatchling sex ratios.
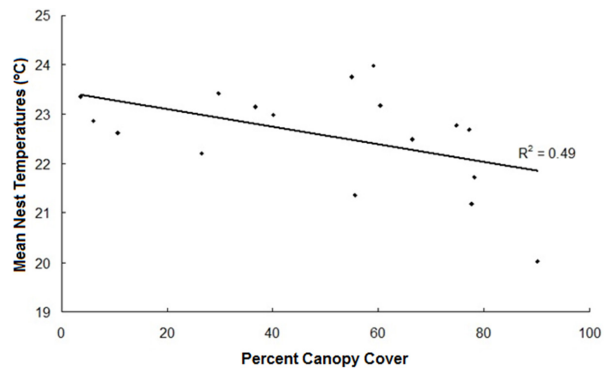
FIGURE 2. Regression of mean nest temperatures (MNT) and percent canopy cover of 17 *C. p. marginata* nests at The Rookery (Geauga County, Ohio). Temperatures were recorded during the thermosensitive period (TSP), and decreased with increasing percent canopy cover from all 4 cardinal directions (N, E, S, W), resulting in a 100% male-biased secondary sex ratio (P = 0.05).

ficing these animals, rather than releasing them into the wild, include (1) the need to document the sex of hatchling turtles by examination of their internal gonads, as the sex of individual hatchlings of *C. picta* cannot be determined by external anatomy (Janzen, 1995); (2) and concerns about releasing unknown zoonotic infections into natural populations after being brought to the laboratory. All specimens were dissected to assess their sex via macroscopic examination of gonads. Males were distinguished definitively by the presence of short gonads, a ductus deferens, and the lack of Müllerian ducts, whereas females were distinguished by elongated gonads and the presence of Müllerian ducts (Bull, 1985; Bull and Vogt, 1979; Girondot et al., 2004; Janzen, 1994). Because it is known that some male specimens will retain nonfunctioning Müllerian ducts, sex was also verified by examination of serially-sectioned gonads (Schwarzkopf and Brooks, 1985; Yntema, 1981).

STATISTICAL ANALYSES

Pearson single linear regressions were used to analyze the following in order to understand the positive or negative correlation acting upon each parameter: (1) canopy cover (average of N, S, E, W) and MNT; (2) and MNT and each locomotor performance measure (swimming sprint speed and righting response). Required assumptions of the regression analyses were confirmed. The alpha value was set at 0.05 to indicate statistical significance using SPSS (SPSS 16.0, PC).

RESULTS

Seventeen *C. p. marginata* nests were monitored during this study; the first nest was laid on 21 May 2009 and the last on 26 June 2009. All nests had a MNT well below the T_{piv} of 28.5°C, with an average of 22.6°C and a range of 20.0–24.0°C during the TSP (Figure 1). These MNT's yielded a 100% male-biased hatchling sex ratio of all nests, which was supported by macroscopic and histological examination of each specimen. Overall, percent canopy cover was inversely and significantly correlated with MNT; however, the relationship was relatively weak ($P = 0.05$, $R^2 = 0.49$, $N = 17$; Figure 2). Neither measure of locomotor performance was significantly correlated with the MNT (sprint speed: $P = 0.37$, $R^2 = 0.05$, $N = 70$; righting response: $P = 0.92$, $R^2 = 0.00$, $N = 70$). The average sprint speed time was 1.66 s with a range of 0.00 to 3.98 s. The average righting response was 31.63 s with a range of 60.0 (failed to right) to 0.88 s.

DISCUSSION

Previous studies have demonstrated that canopy cover can alter nest temperatures (and subsequently the hatchling sex ratio of many turtle species), as well as affect physical fitness among the resultant hatchlings (Janzen, 1995; Weisrock and Janzen, 1999). The fitness parameters used in this study were narrowed down to swimming sprint speed and righting response as indicators of locomotor performance. It is likely that nesting date (i.e., lay date) should have a positive correlation with MNT experienced by each nest. In this study, there was a trend of increasing MNT from the first laid nest (21 May) to the last (26 June; Figure 1). As other studies have illustrated, this led to all of the nests in the study having 100% male hatchlings (Ewert et al., 2004; Janzen, 1994; Raynaud and Pieau, 1985; Vogt and Bull, 1982). This is not surprising given that all of the nests were 4.5–8.5°C below the T_{piv} of 28.5°C during the middle third of incubation (TSP) when sex is determined in

TSD Ia species. In order to assess the population sex ratio, adult turtles were hoop-net trapped during the summer of 2009 (a total of 66 individuals), and via a Schnabel mark-recapture analysis the result was biased towards females at a 2:1 ratio. The observed male-biased hatchling sex ratio in this study, may therefore be an indicator that adult females were able to select nest sites based on temperature differences that would adjust the overall population towards a stable 1:1 male to female sex ratio as suggested by Fisher's Principle (Fisher, 1930). However, according to Freedburg and Wade (2001), there may be culturally transmitted traits that influence offspring behavior (philopatry), even in the absence of maternal influence (i.e., horizontal transmission between unrelated individuals); which may explain the observed female-biased adult population in our study.

It was our assumption that the ability of hatchlings exhibiting TSD Ia to right itself and swim faster will ultimately result in overall increased fitness. There was a broad range of sprinting speeds between cohorts, from multiple individuals never leaving the starting line, to nests in which most finished relatively quickly. Similarly, many hatchlings could not flip right-side-up during the righting response trials; however, some did so very quickly, at less than 1 second. Even though our study lacked statistical significance with respect to locomotor performance, there was a trend towards increased sprint speed and righting response with increases in MNT. Compared with other studies focused on *C. serpentina* and *C. p. bellii*, this occurrence scientifically agrees with the physiological parameters of ectothermic reptiles (Elnitsky and Claussen, 2006; Steyermark and Spotila, 2001).

Quick locomotor responses are fundamental to escaping predators and avoiding desiccation when an individual becomes overturned or stuck while moving to the nearest body of water. There may be a direct link between maternal nest site choice and its influence on hatchling sex ratios and locomotor performance, as there are seasonal differences in ambient temperature that can effectively alter these parameters. Yearly climatic changes and possible transmission of cultural traits may also likely represent major influencing factors for indicating these important differences. Overall, additional seasons of data that can be kept under more controllable conditions are required to enhance the accuracy of any correlations in future research.

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