

EFFECTS OF NATURAL INCUBATION REGIMES ON SEX RATIOS AND FITNESS OF  
HATCHLING MIDLAND PAINTED TURTLES (*CHRYSEMYS PICTA MARGINATA*)

A Thesis Submitted to the  
Office of Graduate Studies  
College of Arts & Sciences of  
John Carroll University  
in Partial Fulfillment of the Requirements  
for the Degree of  
Master of Science

By  
Jason T. Cotter  
2010

**Keywords:** *Chrysemys picta marginata*, hatchling secondary sex ratio, population sex ratio, canopy cover, indirect measures of fitness, pivotal temperature, thermosensitive period

## **Abstract**

The objectives of the current study were to understand how biotic and abiotic factors impact hatchling secondary sex ratios and indirect measures of fitness (i.e., sprint speed and righting time) of *Chrysemys picta marginata* in northeastern Ohio, U.S.A. It was predicted that canopy cover would lower nest temperatures relative to more open nest locations and therefore produce more male-biased hatchling secondary sex ratios, larger hatchlings, and hatchlings with greater indirect fitness measures. Biotic and abiotic factors recorded from each nest were directly correlated with mean nest temperatures, with north facing canopy cover having the largest influence. Additionally, clutch size was directly correlated with percentage of males; however,, mean nest temperatures were not. Male hatchlings were larger than their female cohorts and had faster sprint speeds along a 1-m water-track, whereas the female hatchlings were faster at righting themselves. Overall, this study has illustrated no direct correlation between the amount of canopy cover and hatchling secondary sex ratios as indicated by other studies. This may be due to insufficient variability in ambient temperatures during the course of the study. However, this study did indicate significant variation in the amount of canopy over the nests which affected hatchling righting time, but not hatchling sprint speed.

## **Introduction**

Sex determination has been the focus of much recent research involving reptiles. Specifically, sex determination in reptiles is separated into two broad categories: genotypic and environmental (Bull, 1980; Janzen and Paukstis, 1991a). The mechanisms of genotypic or genetic sex determination (GSD) are those involved in determining the sex of offspring at fertilization, in which the external environment has no influence on the outcome of sex determination (Janzen and Paukstis, 1991b). In contrast, environmental sex determination mechanisms (ESD), such as thermal and hydric properties in the external environment, determine the sex of offspring after fertilization occurs (Janzen and Paukstis, 1991a,b; Janzen, 1995). Snakes, amphisbaenids, and tuataras are known to exhibit GSD; however, all 8 species of crocodylians, some lizards within Sauria, and numerous chelonians exhibit ESD (Janzen and Paukstis, 1991a,b). Specifically, of over 1,000 karyotyped extant reptiles, 354 species are known to exhibit GSD, whereas 72 species exhibit ESD. Additionally, several patterns of ESD and/or GSD have been documented within single subfamilies and genera (Janzen and Paukstis, 1991b).

The most common form of ESD is the temperature-dependent sex determination (TSD) model, in which ambient temperature influences the sex of the embryos after fertilization (Janzen and Paukstis, 1991b). Sex determination in this model is limited to a narrow window of time during the middle third of incubation, known as the thermosensitive period (TSP), and once this period is established, the sex of a particular embryo will be determined (Vogt and Bull, 1982; Janzen, 1994b; Janzen, 1995; Doody, 2004; Ewert et al., 2004; Georges et al., 2004; Rhen and Lang,

2004) (Fig. 1). To infer the TSP for a given reptile, a clutch of eggs must be shifted from an all male- to an all female-producing temperature; therefore, this period apparently can be determined only in a controlled laboratory setting (Janzen and Paukstis, 1991b; Rhen and Lang, 2004).

There are three patterns of TSD that were originally described by Bull (1980) under controlled laboratory conditions: TSD Ia (male-female) produces males at low temperatures and females at high temperatures (most turtle species exhibit this pattern) (Fig. 2A); TSD Ib (female-male) produces females at low temperatures and males at high temperatures (5 crocodylians and apparently most lizards) (Fig. 2B); and TSD II (female-male-female) produces females at the low and high temperature ranges, and only males in the intermediate temperature range (3 crocodylians, and 1 lizard) (Fig. 2C) (Janzen and Paukstis, 1991b; Valenzuela, 2004). Much of the literature, however, is up for debate as to which pattern of TSD various turtle species exhibit because this trait has evolved multiple times.

In all 3 TSD patterns, there is a point known as the pivotal or threshold temperature ( $T_{piv}$ ), at which a 1:1 sex ratio occurs (Vogt and Bull, 1982; Raynaud and Pieau, 1985; Ewert et al., 2004). For example, if the temperature during the middle third of incubation (the TSP) is held at the  $T_{piv}$  for a species with TSD Ia, a 50:50 male:female sex ratio will result and any deviation above or below that temperature will result in a more female- or male-biased sex ratio, respectively (Fig. 2A). The deviation of temperature change influencing sex determination is termed the transitional range (Valenzuela, 2004; Fig. 2B). In species with TSD Ia (e.g.,

*Chrysemys picta*), there is only one transitional range (corresponding to 1  $T_{piv}$ ), which is just over 1.5°C wide (Ewert et al., 2004; Rhen and Lang, 2004) (Fig. 2A,C).

Multiple studies have mapped the evolution of ESD in turtles, and most exhibit either TSD Ia or TSD II (Ewert and Nelson, 1991; Janzen and Paukstis, 1991a,b; Ewert et al., 2004) (Fig. 2A,C; Appendix a). Most research on TSD in turtles has been replicated and supported by numerous laboratory studies focusing on incubation temperature, posthatchling growth rates, embryonic development, and the effects of hormones (Janzen and Paukstis, 1991b; Janzen, 1995; Bowden et al., 2000; Janzen and Morjan, 2002), but relatively few studies have focused on natural field experiments based on temperature alone (Georges et al., 2004; Rhen and Lang, 2004; Place and Lance, 2004). Perhaps one explanation for why much of this work is not performed in natural environments is that numerous variables account for relationships between sex determination and nest temperatures, such as the effects of rainfall, daily temperature fluctuations, seasonal climatic trends, and thermal gradients within the nests (Vogt and Bull, 1982; Georges et al., 2004). For instance, original TSD sex ratio data resulted from controlled laboratory and field experiments, in which one sex was produced above a particular  $T_{piv}$  and the other sex produced below that  $T_{piv}$ . However, it soon became apparent that mean nest temperatures were not the optimal predictor of sex determination (even though it remains in the literature; Janzen, 1994b). Instead, it is the amount of time a particular temperature is held above or below the  $T_{piv}$ , and the proportion of development taking place at those temperatures that serve as the best predictors of hatchling sex ratios (Bull and Vogt, 1979; Bull, 1985; Weisrock and Janzen, 1999; Georges et al., 2004). By knowing the amount of time above or below

the  $T_{piv}$ , the sex ratio can be predicted. For species exhibiting TSD Ia under fluctuating temperatures (e.g., such as incubated under natural conditions with daily or hourly fluctuations in temperature), female turtles will be produced if greater than half of the daily embryonic development is held above the  $T_{piv}$ , whereas males will be produced if greater than half of the daily embryonic development is held below the  $T_{piv}$  (Georges, 1989; Georges et al., 2004). Additionally, as temperatures depart more significantly from the  $T_{piv}$ , the greater the influence of temperature on sex determination (Ewert et al., 2004). Therefore, one should expect that changing abiotic variables (such as variations in daily and seasonal temperature, chance rainfall events and other weather patterns, and shading due to vegetation growing near the nest) could disrupt the amount of time eggs spend above or below a particular  $T_{piv}$ , thereby altering the outcome of sex determination.

With respect to microhabitat variation within geographic locations, localized populations have been found to nest in areas of more or less shade. For instance, *Chrysemys picta* populations in more northern latitudes, such as northern Michigan, do not seek out nesting areas where shading occurs, whereas more southerly populations in Indiana typically seek out nesting sites where shading reduces exposure to solar radiation and warmer temperatures (Janzen and Morjan, 2001; Morjan, 2003; Ewert et al., 2004). These studies illustrate the possibility that females are selecting nest sites based on vegetational cover, and thereby directly influencing the outcome of secondary sex ratios. For example, Map Turtle (*Graptemys ouachitensis* and *G. pseudogeographica*) nests associated with relatively greater vegetation density (and thus cooler temperatures) produced males, whereas nests that were located in open,

sandy areas with little or no vegetation produced females (Vogt and Bull, 1984). It has been hypothesized that females may be able to balance the outcomes of sex ratios in the population as a whole through nest site choice. In support of this hypothesis, a study of *Emys orbicularis* (a species that exhibits TSD Ia) suggested nests subjected to cooler temperatures during the thermosensitive period produced only males, whereas nests that were subjected to higher temperatures during the thermosensitive period produced only females (Pieau, 1982). A study of sex ratios in *Chelonia mydas* and *Lepidochelys olivacea* in Costa Rica has demonstrated that males tend to be produced in shaded nests, whereas females tend to be produced in more open areas (Vogt and Bull, 1982), thereby suggesting that female nest site choice influences secondary sex ratios.

It has been found that vegetational cover over *Chrysemys picta* nests remains relatively stable during the thermosensitive period, which has been suggested as a mechanism of sex determination (Janzen, 1994a). Male-biased sex ratios resulted from nests that were subjected to more vegetational cover, particularly from the south and west, than nests in more open areas (Janzen, 1994a; Morjan and Janzen, 2003). Additionally, a study by Weisrock and Janzen (1999) found that nests of *C. picta* with more south and west vegetational cover were subjected to considerably less time above the  $T_{piv}$  (28.5°C) in July, which resulted in 100% male-biased sex ratios, thereby implying that females may have been selecting nest sites that produced a specific sex. Additionally, with more time spent above 30°C ( $T_{piv}$ ), female hatchlings of Map Turtles (*Graptemys geographica*, *G. ouachitensis*, and *G. pseudogeographica*) were more likely to be produced (Bull, 1985). Overall, these studies indicate that female

nest site choice may have an effect on secondary sex ratios, in which vegetation density influences the outcome of males or females; with males being produced in more shaded nest locations, and females being produced in more open nest locations.

Nest site selection is linked to differences in thermal and hydric regimes. In order for females to choose a site, there must be a thermally- (or hydrically-) patchy environment in which choices can be made for nest site selection and that would allow production of both male and female offspring (Janzen and Paukstis, 1991b). The controversial issue behind maternal nest site choice is whether or not females can predict what temperatures or hydric properties the nests will encounter during embryonic development, and therefore the subsequent sex ratio of the hatchlings (Janzen and Paukstis, 1991b; Janzen, 1994a). Additionally, some authors suggest it needs to be known whether or not females actually return to the exact nesting sites year to year in order to assess if females are selecting nest sites based on microenvironmental properties, such as temperature or individual history and memory of nest sites (Vogt and Bull, 1982; Ewert et al., 2004). In a study of *Chrysemys picta*, adult females nested in shaded areas year to year, although not in the same exact locations (Janzen and Morjan, 2001).

It is known that females nest in both shaded and unshaded areas most likely to counteract the effects of temperature profiles so that all of the nests are not single-sex biased; however, it is unclear if the females are actually selecting these areas non-randomly (Vogt and Bull, 1982; Janzen and Paukstis, 1991b; Ewert et al. 2004). This phenomenon has been corroborated in a *Chrysemys picta* study, in which it was demonstrated that female nest site choice provided no evidence of patterning, and

therefore no indication of an influence on sex ratios due to site-specific characteristics (Schwarzkopf and Brooks, 1987). A likely hypothesis is that some females nest in exclusively female-producing sites, whereas others nest exclusively in male-producing sites (Schwarzkopf and Brooks, 1987). A female could also indirectly choose a nest site based on cohesiveness of the soil, such that the soil does not cave in when the female is digging the nest (Doody, 2004), which may or may not be located in a shaded area. The implications of nest site choice are profound and much recent research has been focused on this phenomenon; however, many questions still remain unanswered.

In contrast, rather than adult females (such as *Chrysemys picta*) selecting nest sites to affect secondary sex ratios, it has been suggested that a stronger argument would be to predict that females are selecting nest sites based on offspring survival (Morjan, 2003). For instance, in an Ontario population of *C. picta*, females consistently chose to nest in relatively warm sites that would increase the probability of successful embryo development, rather than to influence the secondary sex ratio (Schwarzkopf and Brooks, 1987). Similarly, females may select nest sites based on maximizing the developmental rate and the subsequent hatching success of their eggs (Ewert et al., 1994). It has also been suggested that female Striped Mud Turtles (*Kinosternon baurii*), which create shallow nests similar to those of *C. picta*, choose to nest in areas of greater vegetation in order to protect the embryos from thermal extremes (that might increase embryo mortality), rather than to influence the sex ratio (Wilson, 1998).

Temperature has a profound effect on posthatchling growth in *Chrysemys picta*, in which eggs incubated at 30°C (all females) have shown a mass approximately 30% greater than those incubated at 26°C (all males) at one year of age (Janzen and Morjan, 2002). Interestingly, turtles that lay flexible-shelled eggs in relatively cool, wet nest sites produce hatchlings of larger size than those laid in relatively warm, dry nest sites, which is most likely due to the effects on metabolic rate and developmental time (Janzen, 1993). For example, it has been suggested that *Trachemys scripta elegans* hatchlings produced from wetter nests are larger than *T. s. elegans* hatchlings produced from drier nests (Tucker, 2000). Providing evidence for the “bigger is better” hypothesis, larger hatchlings of *Chelydra serpentina* had increased survivorship with respect to growing, running, and swimming faster as compared to smaller individuals (Janzen, 1993). Additionally, a study illustrated that hatchlings from cooler temperatures had faster growth rates than hatchlings produced from warmer temperatures; however, there was no difference between males or females (O’Steen, 1998). *Chelydra serpentina* hatchlings from cooler incubation regimes swam faster and hatchlings from warmer incubation temperatures tended to run more often than stay immobile, implying differences in anti-predator behaviors that might be correlated with differences in fitness potential (Janzen, 1995). Larger *C. picta* females are more likely to produce larger embryos and thus larger hatchlings, thereby increasing the survivorship of the hatchlings (Paitz et al., 2007). Overall, these studies illustrate that adult females may be selecting nest sites based on offspring survival rather than to influence the hatchling secondary sex ratio. It is important to note that posthatching behavior is relatively unknown under natural conditions because

hatchling encounters are quite rare; therefore many fitness studies are lacking early life history characteristics important in understanding this phenomenon (Janzen, 1993).

### **Objectives of Study**

The purpose of this study was to characterize the incubation regimes of naturally-occurring nests of the Midland Painted Turtle (*Chrysemys picta marginata*) and to document the effect on the outcome of sex determination (i.e., hatchling secondary sex ratio) and indirect measures of fitness (e.g., sprint speed and righting time) of hatchlings. I expected that differences in nest site selection by females would have measurable effects on the resulting hatchling secondary sex ratios and indirect measures of fitness, as *C. p. marginata* is known to exhibit TSD Ia temperature-dependant sex determination. I focused on the abiotic (e.g., nest temperature, and distances from water and vegetation) and biotic (e.g., plant communities in which nests are laid) factors that were likely to affect the incubation regime.

I recorded the incubation regimes found within *Chrysemys picta marginata* nests to determine if the microenvironmental differences that arose in each nest could affect both the hatchling secondary sex ratios and the overall fitness of the hatchlings, and thereby alter the overall population structure (e.g., population sex ratio). I tested two hypotheses. First, biotic and abiotic factors will influence the incubation regimes of *C. p. marginata* nests, and therefore the hatchling secondary sex ratio. I predicted that nests with a greater amount of shading would produce clutches with a male-bias in sex ratio, whereas those with less shading would produce clutches with a female-

bias in sex ratio. Second, biotic and abiotic factors will influence the incubation regime of *C. p. marginata* nests, and therefore the indirect measures of hatchling fitness (i.e., sprint speed and righting time). I predicted that nests with a greater amount of shading would produce clutches with larger and faster hatchlings than nests in more open areas.

## **Materials and Methods**

### *Study species*

The Painted Turtle (*Chrysemys picta*) is among the most widespread turtle in North America, extending from British Columbia east to Nova Scotia, south to Georgia, west to Louisiana, north to Oklahoma, and northwest to Oregon (Harding, 1997; Conant and Collins, 1998; Fig. 3). These well-known, small-bodied turtles inhabit quiet, permanent waterways with plenty of basking sites (Harding, 1997). Four distinct subspecies are recognized, but the only one to reside naturally in Ohio is *C. p. marginata*, the Midland Painted Turtle, which generally is distinguished from *C. p. bellii* (Western Painted Turtle), *C. p. picta* (Eastern Painted Turtle), and *C. p. dorsalis* (Southern Painted Turtle) by the presence of a dark, central blotch on the plastron (Harding, 1997). The geographic range of *C. p. marginata* extends from southern Ontario south to southeastern Wisconsin and eastern Illinois to western Kentucky and Tennessee, and eastward to western Virginia, eastern Pennsylvania, and New York (Harding, 1997; Fig. 3).

Like many species of turtles, *Chrysemys picta* exhibits sexual dimorphism in which females are larger than males and adult males possess longer forelimb claws

(relative to the hindlimb claws, as well as relative to those of females) and the position of the cloaca is beyond the posterior margin of the carapace (Gibbons and Lovich, 1990; Gamble and Simons, 2004). Shorter, blunter forelimb claws and an anterior aligned cloaca relative to the posterior margin of the carapace distinguish the females (Gibbons and Lovich, 1990; Gamble and Simons, 2004). Males reach sexual maturity at 3–7 years, whereas females reach sexual maturity at about 6–10 years; both live approximately 35 years in the wild (Harding, 1997). *Chrysemys picta* become active as soon as the ice clears the waterways in its more northern range, generally in late March and early April, and start to display courtship behavior and reproduction under water, typically during April (Harding, 1997). Females lay eggs in mid-May through mid-July throughout their geographic range (Harding, 1997).

Females of *Chrysemys picta* tend to make very shallow (approx. 10-cm deep), flask-shaped nests, which subjects the embryos to differences in ambient temperature during incubation (Janzen, 1994a). Depending on the female, up to 2 clutches per season may be produced, usually within 1–2 weeks of each other (COSEWIC, 2006); however,, some females may not produce a clutch every year (Tinkle et al., 1981; Harding, 1997). Older females tend to produce larger eggs and thus larger hatchlings; however,, clutch size and reproductive frequency do not increase with age (Mitchell, 1988; COSEWIC, 2006). Females of *C. p. marginata* tend to produce no more than 10, 3-cm elliptical, flexible-shelled eggs per clutch (Harding, 1997); however,, clutch size is related to carapace length, the number of clutches produced in a season (1 or 2), and resource acquisition (Rollinson and Brooks, 2008). Most all hatchlings of *C. picta* hatch in late summer through fall (August–October) and overwinter in the nest rather

than emerging immediately (Harding, 1997; Weisrock and Janzen, 1999). Freezing of hatchlings is avoided through supercooling and freeze-tolerance properties (Packard and Packard, 2004; COSEWIC, 2006); the adults hibernate underwater while burrowed into the substrate (Harding, 1997). Since the hatchlings do not exit the nest prior to the following spring, it is possible to obtain an unbiased estimated hatchling secondary sex ratio via excavation of hatched nests in late fall (Janzen, 1994a).

Additionally, this species tends to nest during the hours just before dark, making it possible to observe nesting behavior from a distance (Janzen, 1994a; Harding, 1997).

The thermosensitive-period (TSP) of developing embryos of *Chrysemys picta* occurs in July, specifically between developmental Stages 16 and 22 (Schwarzkopf and Brooks, 1987; Janzen and Paukstis, 1991b). The  $T_{piv}$  of *C. picta* has been recorded in the following ranges: 27.2°C and 28.9°C (Tennessee and North Dakota, respectively; Ewert et al., 2004); 27.0°C and 28.0°C (Tennessee and Indiana, respectively; Ewert et al., 2004); and 28.5°C (Thomson Causeway, IL; Weisrock and Janzen, 1999; Michigan; Ewert et al., 2004).

### *Field Design*

This study was conducted at The Rookery and The West Woods, Geauga County, Ohio, USA (The Rookery: 41°29'59.41"N, 81°17'32.49"W; The West Woods: 41°27'27.82"N, 81°18'13.50"W; Geauga County Park District; Fig. 4) under Ohio Division of Wildlife Wild Animal Permit 10-20 for Scientific Collection and authority of Geauga Park District Regulation #1.1, 4.2, and 9.1 Research Grant. There were 2 major components of this project: (1) determining the population sex ratio of adult *C.*

*p. marginata*, to which secondary sex ratios of the hatchlings would be compared to infer potential changes in population structure over time; (2) and characterizing incubation regimes of natural nests and documenting the effects of nest site selection by females and the incubation regime on hatchling secondary sex ratios and their indirect measures of fitness.

To infer population sex ratios, adult turtles were captured with hoop-net traps from 8 May–5 June, 29 June–2 July, and 14 July–31 July 2009 and analyzed with the Schnabel mark-recapture method (Cox, 2002). These dates were based on both time and funding availability. During the first 18 days, 6 hoop-nets were baited with cantaloupe and tuna or oiled sardines at The Rookery and checked daily to ensure that animals were kept safe (Note: there were numerous instances in which beavers or muskrats chewed holes in the hoop-nets, which may have skewed the results). On 27 May 2009, we began randomly setting 3 of the 6 traps at The West Woods. Each individual turtle was identified by marking the marginal scutes, and from each I assessed sex, mass, length of carapace and plastron, and overall condition of health. If it was a female, its reproductive condition was assessed by palpating for eggs. Upon completion of this procedure, each turtle was placed at the closest point of capture at the water's edge. Basic population sex ratios and demographics were assessed from these data, and all individuals were released at the site of capture. Population size of marked turtles was calculated using the Schnabel mark-recapture method (Cox, 2002).

To assess nest site selection and incubation regimes, 2 field assistants (Kris Carbone and Alynn Martin) and I conducted 49 daily visual surveys of appropriate nesting habitat from 8 May–26 June, 2009 at The Rookery and The West Woods.

This was accomplished by walking along the driveway entrances, trails, off-trail wooded and grassy areas, around all ponds, and through every open area found in the parks everyday with a median of 7.5 hours. Once a turtle was located on land, it was watched from a distance with binoculars to minimize the risk of disturbing it. If the turtle did not nest (i.e., was a male; did not locate an “appropriate” nesting site; and/or was unintentionally disturbed), it was captured by hand and was subsequently assessed for mass, carapace and plastron length, overall condition of health, and if it was a female, its reproductive condition was assessed by palpating for eggs. Additionally, upon confirmation of sex, each female had an approximately 5-cm<sup>2</sup> piece of fluorescent pink tape with its identification number affixed to the posterior section of its carapace to aid further sightings, especially if it did not nest. All turtles were replaced at the point of capture.

Upon completion of a nest, each female was captured and was subsequently assessed for mass, carapace and plastron length, and overall condition of health. Each nest found was carefully probed for presence of eggs, as it is known that females can produce “false-nests” (Tinkle et al., 1981; Harding, 1997). Every nest was fitted with a 46 x 46-cm fine-wire mesh cage over the top to prevent predators from disturbing the eggs (Doody, 1995; Spetz, 2008); each predator-excluder cage was buried approximately 12-cm below the soil and covered the entire nest. A “Please Do Not Disturb” sign and flag were also placed on the cages. At approximately 10-cm from the edge of the cage, a temperature data-logger enclosed within a watertight container with silica-desiccant was buried at approximate middle nest depth to record the incubation regime throughout the season; nests 1–23 had HOBO® TEMP H8

temperature data-loggers, and nests 24–36 had HOBO® U-10 temperature data-loggers following standard protocol for assessing incubation regime (Spetz, 2008). The data-loggers could be accessed without disturbing the incubation regime and temperature data were downloaded to a Palm-V® PDA or laptop computer.

At each nest, I recorded the following variables: lay date; slope; aspect; distance to nearest water; distance from vegetation functional groups (grasses, forbs, trees, and shrubs); percent canopy cover (N, E, S, W); temperature; and clutch size. Slope was found by measuring the steepest side of the nest at the distal end of a 1-m level with the proximal end positioned at the center of the nest. The subsequent calculation of rise over run provided the slope. Aspect of the slope was then taken with a digital compass reading from a GPS. The distance to water variable was assessed by measuring the shortest straight-line distance to the nearest water source from each nest. Additionally, each measure of the vegetation functional groups was based on 3 measurements each of the closest grasses, forbs, trees, and shrubs, which were then pooled and averaged. Percent canopy cover, which included both vegetation and man-made objects, was measured at nest completion, 25 July, and 29 August 2009 using a foresters Model-A spherical densiometer (Janzen, 1994a; Weisrock and Janzen, 1999; Janzen and Morjan, 2001). The densiometer is comprised of a convex mirror with 24, 6-mm<sup>2</sup> sections. By facing the instrument at each respective cardinal direction, I measured the amount of canopy cover reflected from the mirror. Ninety-six total reflections can be measured with respect to each corner of each section (4 corners x 24 mirror sections). Summing the corners that reflect canopy cover and then multiplying each by 1.04 (Janzen, 1994a; Weisrock and Janzen, 1999)

produced proportions of the total canopy cover in relation to each cardinal direction (Janzen, 1994a).

Nest temperature traces were calculated into mean nest temperatures for the middle third of incubation for each nest using HOBOWare® Lite v2.7.2 and HOBOWare® BoxCar Pro v4.3 (Onset Computer Corporation, 2002–2009; Fig. 5). The middle third was calculated as the middle 25 days of a 75 day incubation period for each nest (Justin D. Congdon, personal communication). The mean nest temperatures were then compared to the  $T_{piv}$  of 28.5°C, which allowed for a prediction of the percentage of males in a particular nest. This  $T_{piv}$  is reasonable according to a similar study at nearly the same latitude (Weisrock and Janzen, 1999) as that of my study sites. Even after calculating the mean nest temperatures, there was no difference in estimation of percentage of males with various other  $T_{piv}$ 's, including those not even typical of this longitude and latitude as described in Ewert et al. (1994). Clutch size was determined after excavation of the nests, which was initiated on 27 September 2009 and ended on 7 October 2009, so that the nests were not disturbed during development.

### *Laboratory Design*

There were a total of 38 nests monitored during this study with 30 *Chrysemys picta marginata* (annotated as C.p.\_nest number) and 2 *Chelydra serpentina* (annotated as C.s.\_nest number) nests at The Rookery, and 6 *C. p. marginata* nests at The West Woods. All nests were excavated from 27 September 2009 through 7 October 2009. Upon excavation, 20 of the 30 *C. p. marginata* nests at The Rookery contained live hatchlings and were therefore used in the fitness trials and subsequent analyses under

the approval of the John Carroll University Institutional Animal Care and Use Committee (IACUC). Four of the remaining 10 nests had unhatched eggs that were used in sex determination analyses, whereas the other 6 nests had no viable eggs, and therefore were not included in any statistical analyses. Furthermore, due to low sample sizes, both The West Woods nests (only 4 of the 6 had data), and the *C. serpentina* nests were not used in any statistical analyses.

The number of hatchlings released at the margin of the wetland and the number that was brought back to the laboratory was based upon experimental random sampling by flipping a coin. All hatchlings from 4 nests were kept due to an unexpected early hatching date of 27 September 2009. The following procedure was undertaken for the remaining 34 nests: (1) all hatchlings were brought back to the laboratory for fitness analyses if there were 1-4 total hatchlings in the clutch; (2) if there were 5 total hatchlings in the clutch, 4 were brought back to the laboratory and the fifth individual was released at the margin of the wetland; (3) if there were 6-7 total hatchlings in the clutch, 5 were brought back to the laboratory and the last 2 were released at the margin of the wetland; (4) and if there were 8 or greater total hatchlings in the clutch, half were brought back to the laboratory and the other half released at the margin of the wetland. This procedure made sure that at least one individual would be released (greater than 4 total), yet allowed for adequate replicates. There were 168 total hatchlings (139 *Chrysemys picta marginata*; 29 *Chelydra serpentina*) of which 127 were brought back to the laboratory (105 *C. p. marginata*; 22 *C. serpentina*) in addition to 24 unhatched, yet fully-developed eggs of *C. p. marginata*; thus there were 41 hatchlings released at the sites (4 *C. p. marginata* at The West Woods; 30 *C. p.*

*marginata* at The Rookery; and 7 *C. serpentina* at The Rookery). Four of the 105 *C. p. marginata* hatchlings were not used in the fitness measures because they were experimental trials. The remaining 123 total hatchlings were used in the fitness trials and subsequently sexed; the 24 unhatched *C. p. marginata* eggs were not used in the fitness trials (they were sexed via macroscopic techniques and used in hatchling sex analyses). Due to low replication at The West Woods, only The Rookery *C. p. marginata* data were analyzed and included in this manuscript with the exception of population demographics, which included both sites and both *C. p. marginata* and *C. serpentina* data (see statistical analysis below).

The individuals selected for the fitness trials were transported to Dr. Christopher Sheil's laboratory (John Carroll University) in plastic containers with substrate from the nest. Indirect fitness measures of the hatchlings (sprint speed and righting time trials) were assessed using standard protocols (Janzen, 1993; Janzen, 1995), and used to infer differences in fitness among individuals incubated at various natural incubation regimes. Mass, carapace, and plastron length of these individuals were measured in the laboratory with a bench scale and electronic caliper and then randomly placed into plastic divider boxes for each nest.

Upon completion of these initial measurements, the hatchlings were subjected to a set of 3 sprint speed trials that began on 28 September 2009 and terminated 8 October 2009. Hatchlings from only a few nests were taken to the laboratory for trials and subsequent euthanization on any given day to ensure that individual hatchlings were not kept alive in the laboratory for more than 24 hours (IACUC protocol). The hatchlings were placed into a 10-cm X 2-m water track that was filled with 2.5-cm

20.1°C distilled water and the room was kept at a constant 25°C. The middle of the track contained a 1-m ruler affixed to the bottom. Every hatchling from each nest ran a single trial, which was followed in the same sequence for each subsequent nest. Each hatchling was placed behind the starting line (i.e., 0-m mark), and then gently tapped to stimulate them to swim the track. A screen attached by a hemostat was repeatedly tapped on the track behind the hatchlings without touching them to simulate a predator chasing them. This procedure also prevented them from going backwards in the track. Each hatchling was given 60-sec to complete the 1-m track; the distance covered (point where tip of snout was located) and time was recorded.

From these measures, sprint speed could be calculated by distance (cm) / time (sec) (Note: a 0 constituted no response). Sprint speed trials 2 and 3 were subsequently measured for each hatchling of each nest. Therefore, each hatchling had a rest period of 45–75-min between trials depending on the number of hatchlings in a particular trial period; the 3 trials were pooled and averaged. The righting trials were initiated upon completion of the 3 sprint speed trials. Every hatchling from each nest ran 3 sequential trials on a flat laboratory bench surface with a 15-sec rest between flips, where the hatchling was placed on its back and given a total of 60-sec to complete the trial by returning right side up (Note: For those turtles that did not right themselves within the allotted time, we assigned them a response of 60-sec. This value provides the most conservative estimate for righting time in these individuals). All 3 trials were pooled and averaged.

Upon completion of the fitness trials, all hatchlings were euthanized under humane protocols (standard Institutional Animal Care and Use Committee (IACUC)

by an overdose injection of a 1:1 solution of Nembutal (Na<sup>+</sup> pentobarbital; Janzen, 1994a) into the pericardial cavity, in which *Chrysemys picta marginata* hatchlings received 0.3-mL, and *Chelydra serpentina* received 0.6-mL due to size differences. All specimens were then fixed in 10% buffered formalin, rinsed in three 1-day baths of distilled water, and preserved in 70% ethanol (Janzen, 1994a). The justifications for sacrificing these animals, rather than releasing them into the wild, include: (1) concerns about releasing unknown zoonotic infections into natural populations once animals have been brought into the laboratory; (2) concerns that laboratory experiences may alter natural behaviors; (3) and the need to document sex ratios of hatchling turtles by examination of their internal gonads as sex of individual hatchlings cannot be determined by external anatomy (Janzen, 1995). This phenomenon is found in most other species of reptiles, due to the lack of heteromorphic sex chromosomes; therefore the sex of the individuals is not apparent until near puberty (Ceriani and Wyneken, 2008). Also, I expect that the impact of the loss of these hatchlings on the existing population will be minimal, as very few hatchling turtles survive to sexual maturity due to high predation and mortality (Janzen, 1995; Janzen et al., 2000). In fact, every nest that was caged in this study was tampered with by unknown animals (presumably by predatory raccoons and skunks within the first 24 hours of being laid), and there were 9 *C. p. marginata* nests at The West Woods that were not located prior to their predation (Fig. 6).

All specimens were dissected and assessed for sex, where males were distinguished definitely on the presence of short gonads and a ductus deferens and the lack of Müllerian ducts, whereas females were distinguished by elongated gonads and

the presence of Müllerian ducts via macroscopic examination (Bull and Vogt, 1979; Bull, 1985; Janzen, 1994a; Girondot et al., 2004). This procedure was subsequently verified by histological techniques of the most female- and male-like specimens based on morphology due to many males actually having Müllerian ducts in addition to ductus deferens (Yntema, 1981; Schwarzkopf and Brooks, 1985). Upon completion of this research, the specimens will be accessioned into the vertebrate collection of the Cleveland Museum of Natural History, Cleveland, Ohio, U.S.A.

### *Statistical Analysis*

Only 29 nests of *Chrysemys picta marginata* from The Rookery (Note: nest 6 was removed from all analyses due to spurious temperature data) were included in the following analyses because there were only 4 of 6 viable nests obtained from The West Woods during this study and only 2 *Chelydra serpentina* nests located overall. The 12 nest variables (lay date, slope, aspect, distance to water, distance to forbs, grass, trees/shrubs, N-, E-, S-, W-canopy cover, and clutch size; continuous predictor variables), and mean nest temperatures of the 29 nests (continuous response variable) were analyzed with a multiple linear regression to understand the relationship of the biotic and abiotic factors influencing nest temperatures. To understand the relationship between nest temperatures and the outcome of nest sex ratios, mean nest temperatures of the 29 nests (continuous predictor variable) and the percentage of males that were produced from the nests (continuous response variable) were analyzed using a weighted-least-squares linear regression with clutch size as the weighted variable, due to unequal variance in this variable. Including clutch size in the model

accounts for the fact that the same percentage of males can be observed in nests of different clutch sizes. For instance, a single linear regression would not account for a nest with 100% males with either 1 individual or 5 individuals. Additionally, the amount of canopy cover from all 4 cardinal directions (continuous predictor variable) and percentage of males that were produced from the 29 nests (included as the continuous response variable) were analyzed with a single linear regression to understand the influence of canopy cover acting as shade over the nests and the subsequent sex ratios of those nests. A single linear regression was performed on the carapace length of adult females (continuous predictor variable) and the carapace length of hatchlings (continuous response variable) to understand the relationship between adult female size and hatchling size. Only 16 of the 29 *C. p. marginata* nests at The Rookery were able to be used in this analysis because some adult females could not be measured (i.e., escaped to the water) or the nests had no viable eggs or hatchlings.

In the following analyses (single linear regressions and one-way ANOVA's), only data from the 23 of the 29 nests that had live hatchlings were used. In total, 80 hatchlings were analyzed. Mean nest temperatures of the 23 nests and hatchling size (mass, carapace, and plastron; continuous predictor variables), and indirect hatchling fitness measures (sprint speed and righting time; continuous response variables) were analyzed with separate single linear regressions to understand the relationships between nest temperatures, hatchling size, and indirect hatchling fitness measures, respectively. To understand the relationship between hatchling sex and hatchling sprint speed and righting time, hatchling sex (categorical predictor variable) and the

indirect hatchling fitness measures (continuous response variables) were analyzed with one-way ANOVA's. Hatchling sex (categorical predictor variable) and hatchling size (carapace length; continuous response variable) were analyzed with a one-way ANOVA to understand the relationship between hatchling sex and size. Additionally, hatchling size (mass, carapace and plastron length; continuous predictor variable) and hatchling sprint speed and righting time (continuous response variables) were analyzed with separate single linear regressions to understand the relationship between hatchling size and the indirect hatchling fitness measures. Furthermore, the amount of canopy cover over the nests from all 4 cardinal directions (continuous predictor variable) and carapace length of hatchlings (continuous response variable) were analyzed with a single linear regression to understand the influence of canopy cover acting as shade over the nests and the subsequent size of hatchlings from those nests. Moreover, to understand the relationship between canopy cover acting as shade over the nests and the indirect hatchling fitness measures, canopy cover from all 4 cardinal directions (continuous predictor variable) and hatchling sprint speed and righting time (continuous response variables) were analyzed with separate single linear regressions. Population demographics of the two sites, including both *Chrysemys picta marginata* and *Chelydra serpentina* trapping and hand capture data were analyzed using the Schnabel mark-recapture method (Cox, 2002), and simple sex ratio calculations of the adult and hatchling populations.

The output from all analyses were checked for and met the required regression and ANOVA assumptions. The alpha value was set at 0.05 to indicate statistical significance. I used SPSS© v16.0 to analyze all my data.

## Results

### *Hoop-net Trapping*

Overall, there was approximately 845 field research hours with 150 hours involving trapping and 637 hours (median of 7.5 hours daily) involving nest searching between all researchers in this study (Table 1). The remaining time consisted of installing predator-excluder cages and temperature data-loggers, as well as estimating canopy cover and measuring or recording the nest variables (Table 1). During this study 101 adult turtles were trapped with hoop-nets or captured by hand, including *Chrysemys picta marginata* and *Chelydra serpentina*; even though this study intended to capture only *C. p. marginata* (Tables 2A,B). These captures demonstrated a 2:1 F:M (female:male) *C. p. marginata* ratio at The Rookery with 10 and 1 females recaptured by hand and hoop-net traps, respectively, in addition to 3 and 0 males recaptured with hoop-net traps and by hand, respectively (Tables 2A,B). There was a 5:1 F:M *C. p. marginata* ratio at The West Woods with 9 and 1 females recaptured by hand and hoop-net trap, respectively, and no recaptures of males (Tables 2A,B). There was a 1:1 F:M *C. serpentina* ratio at The Rookery with 3 and 0 females recaptured with hoop-net traps and by hand, respectively, and 3 male recaptures with hoop-net only (Note: 2 of the male recaptures were the same individual; Tables 2A,B). With respect to *C. serpentina* at The West Woods, only 1 female was captured by hand with no recapture thereafter (Tables 2A,B). In addition to these captured turtles, there were a total of 9 sightings of *Clemmys guttata* (1 unidentified individual according to a Geauga Park District, Ohio on-going study), and 1 sighting of *Emydoidea blandingi* at The Rookery.

A Schnabel mark-recapture analysis (Cox, 2002) indicated there was at the time of trapping, an estimated population of 118 adult *Chrysemys picta marginata* at The Rookery (SE  $\pm$  0.00; variance = 0.00). However, according to hand captures, there was an estimated population of 71 adult *C. p. marginata* (SE  $\pm$  0.00; variance = 0.00). Additionally, there was an estimated population of 32 adult *Chelydra serpentina* at The Rookery with respect to trap captures (SE  $\pm$  0.00; variance = 0.00); however, an infinite population estimation based on hand captures because there were no recaptures by hand. At The West Woods, there was an estimated infinite adult *C. p. marginata* population with respect to trap captures because no recaptures were made; however,, there was an estimated population of 17 individuals according to hand captures (SE  $\pm$  0.00; variance = 0.00). According to visual estimates, we saw over 40 adult *C. p. marginata* at The West Woods. Additionally, there was an estimated infinite adult *C. serpentina* population at The West Woods due to no recaptures.

### *Nests*

Overall, there were 36 *Chrysemys picta marginata* (30 at The Rookery; 6 at The West Woods), and 2 *Chelydra serpentina* (The Rookery) nests located, caged, and monitored during this study (Fig. 6 and 7). There were 2 female *C. p. marginata* that produced double clutches at The Rookery. One female laid the first and second clutch on 1 June and 25 June, respectively, and the other female laid the first and second clutch on 4 June and 24 June, respectively (Fig. 7). After excavating the nests in September and October, I found all eggs of 8 different *C. p. marginata* nests dead (6 at

The Rookery; and 2 at The West Woods) (Note: these nests were used in the nest variable analyses with mean temperatures experienced in those nests). An additional 3 *C. p. marginata* nests at The Rookery (a double-clutched nest) had eggs that were very close to being hatched (Note: all eggs were predicted to have hatched at least 34 days prior to being excavated), and therefore used in determining sex of hatchlings and the subsequent analyses that included information about percent males, but not the fitness trial analyses. Even though soil type and percent water content was not measured in this study, I did observe that nests laid in mulch and/or clay soils did not have any viable eggs with the exception of one nest at each site (The Rookery: only 1 live hatchling and 10 dead eggs; The West Woods: all hatchlings alive and no dead eggs). With respect to hatchling secondary sex ratios, there was a 1:15 F:M *C. p. marginata* and a 3:1 F:M *C. serpentina* hatchling secondary sex ratio at The Rookery, and a 1:7.5 F:M *C. p. marginata* hatchling secondary sex ratio at The West Woods. Overall nest survivorship of The Rookery *C. p. marginata* population was 58.54%, whereas I calculated a 54.29% nest survivorship for the *C. p. marginata* population at The West Woods, and 78.38% nest survivorship for the *C. serpentina* population at The Rookery.

As indicated by a multiple linear regression, mean nest temperatures (Fig. 8) were significantly influenced by the other 12 nest variables, in which north canopy cover (NCC) had the largest overall influence followed by lay date, south canopy cover (SCC), slope, east and west canopy cover (ECC, WCC), distance to water, distance to forbs, aspect, distance to grasses, and distance to trees/shrubs ( $F_{(12,16)} = 4.15$ ;  $P = 0.005$ ;  $N = 29$ ; Table 3). Mean nest temperatures had no effect on the

percentage of males; however, there was an effect of clutch size on percent males, in which increases in clutch size indicated a lower percentage of males of the 29 *C. p. marginata* nests at The Rookery ( $F_{(2,20)} = 6.6$ ;  $P = 0.006$ ;  $N = 29$ ; Table 4). The amount of canopy cover over the nests from all 4 cardinal directions was not significantly correlated with the percentage of males that were produced from the nests ( $F_{(1,21)} = 2.21$ ;  $P = 0.15$ ;  $N = 29$ ; Fig. 9).

Hatchling carapace length significantly increased with adult female carapace length ( $F_{(1,68)} = 32.8$ ;  $P < 0.0001$ ;  $N = 70$ ; Fig. 10). However, hatchling size was not significantly correlated with mean nest temperatures (mass:  $F_{(1,78)} = 0.28$ ;  $P = 0.59$ ; carapace length:  $F_{(1,78)} = 0.02$ ;  $P = 0.88$ ; plastron length:  $F_{(1,78)} = 0.11$ ;  $P = 0.75$ ;  $N = 80$ ; Fig. 11A,B). Furthermore, the amount of canopy cover over the nests from all 4 cardinal directions was not significantly correlated with hatchling carapace length ( $F_{(1,78)} = 1.73$ ;  $P = 0.19$ ;  $N = 80$ ; Fig. 12). However, hatchling righting time significantly increased with the amount of canopy cover over the nests from all 4 cardinal directions, but hatchling sprint speed did not (sprint speed:  $F_{(1,78)} = 2.09$ ;  $P = 0.15$ ;  $N = 80$ ; Fig. 13; righting time:  $F_{(1,78)} = 6.75$ ;  $P = 0.01$ ;  $N = 80$ ; Fig. 14). Both measures of fitness were not significantly correlated with mean nest temperatures (sprint speed:  $F_{(1,78)} = 0.00$ ;  $P = 0.96$ ;  $N = 80$ ; righting time:  $F_{(1,78)} = 1.43$ ;  $P = 0.24$ ;  $N = 80$ ; Fig. 15A,B). Male *C. p. marginata* hatchling carapace length was larger than for females by a difference of 0.832-mm ( $F = 9098.9$ ;  $P < 0.0001$ ;  $N = 80$ ; male SE  $\pm 0.20$ ; female SE  $\pm 0.24$ ; Fig. 16). Furthermore, hatchling *Chrysemys picta marginata* males were significantly faster with respect to sprint speed along the 1-m track

distance than were hatchling *C. p. marginata* females by a difference of 0.202-seconds ( $F = 95.8$ ;  $P < 0.0001$ ;  $N = 80$ ; male SE  $\pm 0.12$ ; female SE  $\pm 0.63$ ; Fig. 17).

Hatchling female *Chrysemys picta marginata* were significantly faster at righting themselves compared to the hatchling male *C. p. marginata* with a difference of 9.39-seconds ( $F = 80.0$ ;  $P < 0.0001$ ;  $N = 80$ ; male SE  $\pm 2.67$ ; female SE  $\pm 8.67$ ; Fig. 18). Additionally, there were no significant differences between hatchling mass, carapace length, or plastron length and sprint speed (mass:  $F_{(1,78)} = 0.55$ ;  $P = 0.46$ ; carapace length:  $F_{(1,78)} = 3.04$ ;  $P = 0.09$ ; plastron length:  $F_{(1,78)} = 3.50$ ;  $P = 0.07$ ;  $N = 80$ ; Fig. 19A,B), or righting time (mass:  $F_{(1,78)} = 0.79$ ;  $P = 0.38$ ; carapace length:  $F_{(1,78)} = 1.68$ ;  $P = 0.20$ ; plastron length:  $F_{(1,78)} = 2.54$ ;  $P = 0.12$ ;  $N = 80$ ; Fig. 20A,B).

## Discussion

### *Hatchling secondary sex ratios*

The 12 nest variables (lay date, slope, aspect, distance to water, distance to forbs, grass, trees/shrubs, NCC, ECC, SCC, WCC, and clutch size) measured during this study were positively correlated with mean nest temperatures of *Chrysemys picta marginata* at The Rookery (Table 3). Most influential was the NCC ( $P = 0.033$ ) followed by lay date ( $P = 0.057$ ), in which the former has not been found in other studies (Janzen, 1994a; Weisrock and Janzen, 1999). South canopy cover and WCC have been shown in previous studies to be most influential in determining nest temperatures, especially during the month of July (Janzen, 1994a, Weisrock and Janzen, 1999). Nests with more canopy cover from the west and south typically produced a more male-biased sex ratio than did the more exposed nests because that

canopy cover shaded much of the solar radiation and lowered nest temperatures (Janzen, 1994a). It is clear that lay date should have a positive correlation with mean nest temperatures experienced by nests, since temperatures steadily increased throughout the incubation period (i.e., May–August). In this study, there was a trend of increasing mean nest temperatures from the first laid nest (21 May) to the last laid nest (26 June; Fig. 8); however, lay date was not statistically significant. The mean nest temperatures were not significantly correlated with the percentage of males; however, clutch size indicated a decrease in the percentage of male hatchlings, which means there would be a greater chance of females having been produced in larger clutches (Table 4). Overall, females were rare in this study, so it is not surprising that the probability of a clutch producing a female increased with clutch size. According to other studies, fluctuating nest temperatures have been found to be directly correlated with offspring sex ratios (Schwarzkopf and Brooks, 1985; Georges et al., 1994); however, this relationship was not found in this study. I attribute this to minimal temperature variation among nests, which may have contributed to the failure to detect any significant changes in hatchling secondary sex ratios caused by mean nest temperatures. However, the finding of clutch size having a significant influence on the percentage of males has not been indicated in other studies to my knowledge. This may indicate that clutch size also has a large influence on hatchling secondary sex ratios, rather than just nest temperatures as indicated in other studies (Janzen, 1994a; Weisrock and Janzen, 1999).

Every nest studied had a mean nest temperature well below the  $T_{piv}$  of 28.5°C with a range of 20.02–24.03°C, which in *Chrysemys picta marginata* should

correspond to a 100% male-biased sex ratio. What is interesting is that there were 7 females produced from 4 different nests. Of these nests, only one had relatively little canopy cover, and therefore was predicted to have produced females as other studies have illustrated (Janzen, 1994a, Weisrock and Janzen, 1999). On the other hand, the nests that were subjected to relatively more canopy cover were predicted to produce a 100% male-biased sex ratio as indicated by other studies (Janzen, 1994a, Weisrock and Janzen, 1999), yet they produced some females. For instance, one of the females that produced a double clutch, laid her first clutch in an almost 0% canopy cover location; however,, laid her second clutch in an almost 100% canopy cover location, yet both nests produced a 100% male-biased sex ratio, with the sex of the most female-like individuals verified by histological techniques (Yntema, 1981; Schwarzkopf and Brooks, 1985). One explanation for this phenomenon is that the females produced from these nests were from the eggs at the very top of the nests, and therefore were subjected to warmer, more female-producing temperatures. One flaw in this study may have been the placement of the temperature data-loggers. It was not feasible to place temperature probes into the nests due to a lack of funding, and therefore the data-loggers were buried next to the nests at approximately mid-nest level. Another explanation is that not all hatchlings were taken for sex identification. Even though there was a trend towards nests being 100% male-biased with NCC being most influential in terms of correlation with mean nest temperatures, there was no clear trend between the amount of canopy cover and the subsequent percent male hatchlings of *C. p. marginata* ( $P = 0.152$ ; Fig. 9). Overall, this study did illustrate that there were differences in incubation regimes between nests due to biotic and abiotic

factors; however,, these findings are not consistent with my first hypothesis, suggesting that there was no difference between the amount of shading caused by canopy cover and the subsequent hatchling secondary sex ratios. A likely explanation is that the small number of females sampled may have contributed to the failure to detect any significant changes in the amount of canopy cover and the subsequent hatchling secondary sex ratios. Additionally, the 2009 nesting season was relatively cool, which may not have allowed nests to experience female-producing temperatures.

In the current study, I cannot state that there was any female nest site choice due to the short period of time this study was conducted. Furthermore, I cannot infer that females were able to “predict” nest temperatures that the incubation period would experience, as indicated by Janzen (1994a). However, studies have suggested that adult female turtles are able to select nest sites based on microenvironmental cues (Janzen and Morjan, 2001; Morjan, 2003; Ewert et al., 2004); however, other studies have questioned if females can predict what temperatures or hydric properties the nests will encounter during embryonic development, and therefore the subsequent sex ratio of the hatchlings (Janzen and Paukstis, 1991b; Janzen, 1994a). In an Ontario population of *Chrysemys picta*, female nest site choice provided no evidence of patterning nest site selection, and therefore no indication of an influence on sex ratios due to site-specific characteristics (Schwarzkopf and Brooks, 1987). One of the likely hypotheses is that some females nest in exclusively female-producing sites, such as open areas, whereas others nest exclusively in male-producing sites, such as highly vegetated areas (Schwarzkopf and Brooks, 1987). The implications of nest site choice are profound and much recent research has been focused on this phenomenon (Vogt

and Bull, 1982; Schwarzkopf and Brooks, 1987; Janzen and Paukstis, 1991b; Ewert et al., 2004), however many questions still remain unanswered. In order to fully understand the implications of maternal nest site choice, future studies must focus efforts on conducting long-term observations of known marked populations in order to elucidate whether or not females are actually selecting nest site microenvironments based on those microenvironmental cues.

#### *Indirect measures of hatchling fitness*

Vegetational cover not only alters nest temperatures, and subsequently the secondary sex ratio of many turtle species, it also affects proxies of fitness among the resultant hatchlings (Weisrock and Janzen, 1999), and therefore, much recent research has been focused on this topic, particularly in turtles (Janzen, 1993; Janzen, 1995). As indicated previously, all *Chrysemys picta marginata* nests at The Rookery were well below the  $T_{piv}$  of 28.5°C, and therefore there was not a robust sample size of both male- and female-producing temperatures, even though 4 nests did produce a total of 7 females. There was no significant relationship among mean nest temperatures, hatchling size, or indirect measures of fitness (i.e., sprint speed and righting time) of *C. p. marginata* hatchlings at The Rookery (Fig. 11A,B and 15). This was most likely caused by low mean nest temperatures among all nests. Furthermore, the amount of canopy cover over the nests from all 4 cardinal directions was not significantly correlated with hatchling carapace length ( $P = 0.19$ ; Fig. 12). Additionally, there was no significant difference between hatchling mass, carapace and plastron length and that of their sprint speeds and righting times (Fig. 19A,B; 20A,B). These findings are

not consistent with my second hypothesis, suggesting that there was not sufficient variation in the amount of canopy cover over the nests to affect the size of the hatchlings. A likely explanation is that there was minimal variation in the amount of canopy cover over nests as well as a low sample size that may have contributed to the failure to detect changes in hatchling size. However, I did find significant variation among the amount of canopy cover acting as shade and that of hatchling righting time, but not hatchling sprint speed ( $P = 0.01$ ; Fig. 14;  $P = 0.15$ ; Fig. 13). These findings suggest variation in canopy cover may influence some indirect measures of fitness. I attribute the differences between hatchling sprint speed and righting time to the size differences between the hatchlings. The hatchlings that righted faster were smaller, and therefore more likely to flip over quicker because of a shorter pivoting point (i.e., the perpendicular distance of the carapace to the flat lab bench).

Other studies have found that fluctuating nest temperatures do affect the size of hatchling turtles (Janzen, 1993, Janzen and Morjan, 2002). Temperature can have a profound effect on posthatchling growth in *Chrysemys picta*; in one study, eggs incubated at 30°C (all females) were approximately 30% heavier than those incubated at 26°C (all males) at one year of age (Janzen and Morjan, 2002). This study illustrated a mean carapace length difference between male and female hatchlings of 0.832-mm, in which the males were the slightly larger individuals ( $P < 0.0001$ ; Fig. 16). Additionally, the male hatchlings in this study had faster sprint speeds than their female cohorts with a difference of 0.202-s ( $P < 0.0001$ ; Fig. 17). This may suggest that larger individuals are faster than smaller individuals, suggesting a biological significance with respect to escaping predation. On the other hand, female hatchlings

were faster at righting themselves than their male cohorts separated by a time of 9.39-s ( $P < 0.0001$ ; Fig. 18), which may suggest a biologically significant difference with respect to hatchling fitness due to a smaller carapace on which the hatchlings pivot themselves to flip over (i.e., a shorter perpendicular distance with respect to a substrate).

It was suggested that an approximate 0.63-mm difference between male and female hatchling *Chelydra serpentina* (in which the females had shorter plastrons than their male cohorts) probably does not illustrate any biological significance (Janzen, 1995). However, other authors suggest seemingly small differences in length can have large impacts on overall body size (e.g., total volume) and thus can have important demographic influences on turtle populations (Tucker, 2000). Furthermore, a 0.8-mm and a 0.33-g difference in carapace length and carcass mass of *Trachemys scripta elegans*, respectively, was suggested to have ecological credibility in terms of physiological responses of turtle embryos to biotic factors (i.e., water potential; Tucker et al., 1998).

Focusing on *Chelydra serpentina*, the “bigger is better” hypothesis has suggested that natural selection favors larger hatchlings of *C. serpentina* because they have increased survivorship with respect to growing, running, and swimming faster as compared to smaller individuals, and therefore are more able to escape predation and starvation (Janzen, 1993; Janzen, 1995). It is suggested that this may imply differences in anti-predator behaviors that might be correlated with differences in fitness potential (Janzen, 1995; Girondot et al., 2004). This has been corroborated in a *Chrysemys picta* study, where longer hatchlings were faster than shorter hatchlings

(Paitz et al., 2007). However, as indicated previously, this study has demonstrated that hatchling size (i.e., carapace length) and indirect measures of fitness (i.e., sprint speed and righting time) of *C. p. marginata* were not significantly correlated, suggesting there is no biological significance between these attributes. Overall, there was no clear relationship between nest site characteristics and the fitness of the *C. p. marginata* hatchlings from The Rookery, which is most likely attributed to a very low number of female hatchling replicates. With relatively few studies (Janzen, 1993; Janzen, 1995) focusing on differences between hatchling sexes, there are many uncertainties on the actual biological significance of size measures between individuals, and therefore more long-term studies need to focus on these questions to fully understand the implications of male and female fitness (Tucker, 2000). I did, however, find a significant increase in the carapace length of *C. p. marginata* hatchlings with increases in adult female carapace length at The Rookery ( $P < 0.0001$ ; Fig. 10). Similar results were found in a *C. picta* study in which larger females were more likely to produce larger embryos, and thus larger hatchlings; potentially increasing hatchling survivorship (Paitz et al., 2007).

According to the mark-recapture analysis conducted during this study, there was an apparently healthy population of *Chrysemys picta marginata* at The Rookery with an estimated population of 118 adult individuals with a 2:1 F:M ratio. It has been proposed that populations are likely to shift towards equilibrium of males and females in order for a population of turtles to remain stable (Schwarzkopf and Brooks, 1987; Janzen, 1995). Therefore, it is possible that The Rookery *C. p. marginata* population is undergoing this phenomenon; however, it is unclear due to only one field season of

data. Multiyear data sets are imperative to fully understand the sex ratios of species that exhibit TSD (Girondot et al. 2004). Additionally, under the female dominance phenomenon, it has been suggested that the number of juveniles of a particular sex (i.e., secondary sex ratio) produced in a population is directly correlated with the number of sexually mature females, while it is also suggested that a low number of males may in fact be the limiting factor in reproductive events (Girondot et al., 2004). After analyzing the overall secondary sex ratio of this population, it became clear that the 2009 reproductive season may have aided in this equilibrium process. The secondary sex ratio was over 90% male-biased, which may indicate that females were “selectively-choosing” nest sites that would tend to shift the population sex ratio towards a 1:1 ratio. It could also be that the 2009 nesting season was just too cool to produce a large number of females.

Throughout this study there were several confounding factors that may have led to a lack of significance of the overarching objectives and hypotheses of this study. In addition to those already mentioned, it was difficult to acquire actual canopy cover estimates with the spherical densiometer at each nest because the predator excluder cages prevented any estimation of direct canopy cover over the nest center, such as grasses that grow in and within the cages. The cages were not removed to estimate canopy cover because they would have disturbed the incubation regime and would have added a significant amount of time and energy to remove and replace. Additionally, a factor that I could not control, was the amount of movement that overhanging vegetation imposed upon the reading and interpretation of the spherical densiometer. Furthermore, there were other variables that would likely have attributed

largely to the outcome of both nest temperature data and thus secondary sex ratios and indirect measures of hatchling fitness that I was unable to measure. For instance, elevation above the water was unattainable due to the majority of nests being located over 100-m from the nearest water source. Soil moisture-content of the nests would also have aided in both hatchling size and sex difference analyses, which may influence the overall indirect measures of hatchling fitness in this population. Additionally, nest depths were unattainable due to the nests either completely breaking apart as I removed the predator-excluder cages to excavate the nests or because I had to completely scrape away the entire top surface of the nests in order to reach the hatchlings without harming them. The correlation between ambient temperature and nest temperature would also have aided in the determination of percentage of males produced from a nest (Janzen, 1994b). Additionally, by initiating both water- and substrate-track trials along with righting trials on various substrates would help to better understand the relationship between hatchling size, sex, and indirect measures of fitness. It would be beneficial for a future study to incorporate these variables and parameters to better understand the influence of biotic and abiotic factors acting upon secondary sex ratios and indirect measures of fitness of hatchling turtles.

Better control of these confounding factors would likely have provided more robust results in the current study, especially those involving the placement and use of temperature data-loggers and temperature probes. Additionally, future studies involving the nesting behavior, nest characteristics, and the subsequent hatchling secondary sex ratio and indirect fitness measures of not only The Rookery *Chrysemys picta marginata* population, but other turtle studies as well, need to incorporate a

longer time frame (i.e., 5 or more years), as well as the ability to acquire greater funding to accurately assess these measures of natural incubation. It is imperative in the years to come to more fully understand the implications of how various biotic and abiotic factors influence not only maternal nest site choice, but how varying climatic patterns may ultimately affect the population demographics of species that exhibit TSD.

This study should provide Geauga Park District an overall better understanding of population structure and demographics of turtle species at The Rookery and The West Woods, particularly that of *Chrysemys picta marginata* and *Chelydra serpentina*. For instance, all individuals of all turtle species that were caught and/or observed at the study sites were recorded (i.e., sex, mass, carapace and plastron length, location, and reproductive fitness) and marked to establish baseline data on population structure (with the exception of *Clemmys guttata* and *Emydoidea blandingi*, as these are protected species). The potential impact of vegetation acting as shade over the nests on incubation regimes and the subsequent hatchling secondary sex ratios and fitness of *C. p. marginata* were studied thoroughly at The Rookery, which may provide insight into vegetation management for the protection of this species in the future (i.e., elimination of invasive, non-native plant species). This study has demonstrated that there was no significant difference between nests that had more or less canopy cover, and therefore the issue of mowing and landscaping does not seem to have an effect on the *C. p. marginata* population at The Rookery. I do recommend that visitors of the Geauga County park system are notified of the nesting seasons (i.e., May through June) and behaviors of the turtle species found in the parks (with the exception of *E.*

*blandingi* and *C. guttata* because these are protected species); specifically stressing that turtles are not to be disturbed. For instance, I observed on several occasions female *C. p. marginata* returning to the water after being disturbed while trying to locate appropriate nesting sites, as well as two gravid adult female *C. p. marginata* killed by cars on The Rookery driveway (one was a marked female with pink tape). Additionally, I recommend a predator-control program that either relocates or terminates potential predators in the parks because there were 9 *C. p. marginata* nests predated at The West Woods, and all nests that were affixed with predator-excluder cages in this study were tampered with by unknown predators.

## **Acknowledgements**

I would like to, first and foremost, thank my parents and my beautiful girlfriend Natanya for their love, support, and help throughout this research. I would also like to thank Dr. Erik Wild for getting me so involved with herpetofauna and ultimately referring me to Dr. Christopher Sheil who has become not only my graduate advisor, but a friend as well. I want to thank him for his expertise in the field of herpetology and the immense help that he has provided me throughout my graduate career. I also want to thank the biology department of John Carroll University for the opportunity to undergo graduate studies with provided funding for my research. Within the biology faculty, I want to thank my committee members, Drs. Carl Anthony and Rebecca Drenovsky for their invaluable advice and help with the implementation of this research. I would also like to thank Kris Carbone and Alynn Martin for their excellent, enthusiastic, very hard-working ethics in the field and laboratory research aspects of this project. If it wasn't for Kris, we wouldn't have found the first *C. p. marginata* nest! I want to thank Geauga Park District for granting me with a generous amount of funding to conduct this research in the park system, and I especially want to thank Paul Pira and Dan Best for their help in implementing this research. I would also like to thank all of The Rookery and The West Woods visitors for their help in providing turtle and nest locations throughout the summer, especially the very nice JCU graduate who was out at The Rookery every night with her children.

## Literature Cited

- Bowden, R. M., M. A. Ewert, and C. E. Nelson. 2000. Environmental sex determination in a reptile varies seasonally and with yolk hormones. *The Royal Society* 267:1745–1749.
- Bull, J. J. 1980. Sex determination in reptiles. *The Quarterly Review of Biology* 55:3–21.
- . 1985. Sex ratio and nest temperature in turtles: comparing field and laboratory data. *Ecology* 66:1115–1122.
- Bull, J. J., and R. C. Vogt. 1979. Temperature-dependent sex determination in turtles. *Science* 206:1186–1188.
- Ceriani, S. A., and J. Wyneken. 2008. Comparative morphology and sex identification of the reproductive system in formalin-preserved sea turtle specimens. *Zoology* 111:179–187.
- Conant R., and J. T. Collins. 1998. *A Field Guide to Reptiles and Amphibians: Eastern and Central North America*. 4<sup>th</sup> Ed. The Peterson Field Guide Series. New York: Houghton Mifflin. 616 p.
- COSEWIC. 2006. Committee on the Status of Endangered Wildlife in Canada assessment and status report on the Western Painted Turtle *Chrysemys picta bellii* (Pacific Coast population, Intermountain-Rocky Mountain population and Prairie/Western Boreal-Canadian Shield population) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vii + 40 pp.
- Cox, G.W. 2002. Mark-recapture and catch-effort population estimates. Pp. 113–123. *General Ecology Laboratory Manual* (8<sup>th</sup> edition). McGraw Hill., New York.
- Doody, J. S. 1995. A comparative nesting study of two syntopic species of softshell turtles (*Apalone mutica* and *Apalone spinifera*) in southcentral Louisiana. Thesis (M.S.)-Southeastern Louisiana University, Hammond, LA.
- . 2004. Determinants of reproductive success and offspring sex in a turtle with environmental sex determination. *Biological Journal of the Linnean Society* 81:1–16.
- Ewert, M. A., and C. E. Nelson. 1991. Sex determination in turtles: diverse patterns and some possible adaptive values. *Copeia* 1991:50–69.

- Ewert, M. A., D. Jackson, and C. E. Nelson. 1994. Patterns of temperature-dependent sex determination in turtles. *Journal of Experimental Zoology* 270:3–15.
- Ewert, M. A., C. R. Etchberger, and C. E. Nelson. 2004. Turtle sex-determining modes and TSD patterns, and some TSD pattern correlates. Pp. 21–32. In N. Valenzuela and V. A. Lance (Eds.), *Temperature-dependent Sex Determination in Vertebrates*. Smithsonian Institution Press., Washington D.C.
- Gamble, T., and A. M. Simons. 2004. Comparison of harvested and nonharvested painted turtle populations. *Wildlife Society Bulletin* 32:1269–1277.
- Geauga Park District. 2008. Map of Geauga Park District's parks and preserves. Chardon, OH.
- Georges, A. 1989. Female turtles from hot nests: is it duration of incubation or proportion of development at high temperatures that matters? *Oecologia* 81:323–328.
- Georges, A, S. Doody, K. Beggs, and J. Young. 2004. Thermal models of TSD under laboratory and field conditions. Pp. 79–89. In N. Valenzuela and V. A. Lance (Eds.), *Temperature-dependent Sex Determination in Vertebrates*. Smithsonian Institution Press., Washington D.C.
- Gibbons, J. W., and J. E. Lovich. 1990. Sexual dimorphism in turtles with emphasis on the slider turtle (*Trachemys scripta*). *Herpetological Monographs* 4:1–29.
- Girondot, M., V. Delmas, P. Rivalan, F. Courchamp, A. C. Prévot-Julliard, and M. H. Godfrey. 2004. Implications of temperature-dependent sex determination for population dynamics. Pp. 148–155. In N. Valenzuela and V. A. Lance (Eds.), *Temperature-dependent Sex Determination in Vertebrates*. Smithsonian Institution Press., Washington D.C.
- Harding, J. H. 1997. *Amphibians and Reptiles of the Great Lakes Region*. Ann Arbor: The University of Michigan Press. 378 p.
- Janzen, F. J. 1993. An experimental analysis of hatchling selection on body size of hatchling turtles. *Ecology* 74:332–341.
- . 1994a. Vegetational cover predicts the sex ratio of hatchling turtles in natural nests. *Ecology* 75:1593–1599.
- . 1994b. Climate change and temperature-dependent sex determination in reptiles. *Proceedings of the National Academy of Sciences* 91:7487–7490.
- . 1995. Experimental evidence for the evolutionary significance of temperature dependent sex determination. *Evolution* 49:864–873.

- Janzen, F. J., and G. L. Paukstis. 1991a. A preliminary test of the adaptive significance of environmental sex determination in reptiles. *Evolution* 45:435–440.
- . 1991b. Environmental sex determination in reptiles: ecology, evolution, and experimental design. *The Quarterly Review of Biology* 66:149–179.
- Janzen, F. J., and C. L. Morjan. 2001. Repeatability of microenvironment-specific nesting behaviour in a turtle with environmental sex determination. *Animal Behaviour* 62:73–82.
- . 2002. Egg size, incubation temperature, and posthatching growth in painted Turtles (*Chrysemys picta*). *Journal of Herpetology* 36:308–311.
- Janzen, F. J., J. K. Tucker, and G. L. Paukstis. 2000. Experimental analysis of an early life-history stage: selection on size of hatchling turtles. *Ecology* 81:2290–2304.
- Mitchell, J. C. 1988. Population ecology and life histories of the freshwater turtles *Chrysemys picta* and *Sternotherus odoratus* in an urban lake. *Herpetological Monographs* 2:40–61.
- Morjan, C. L. 2003. Variation in nesting patterns affecting nest temperatures in two populations of painted turtles (*Chrysemys picta*) with temperature-dependent sex determination. *Behavioral Ecology and Sociobiology* 53:254–261.
- Morjan, C. L., and F. J. Janzen. 2003. Nest temperature is not related to egg size in a turtle with temperature-dependent sex determination. *Copeia* 2003:366–372.
- Onset Computer Corporation. 2002. HOBOboxCar® Pro version 4.3. Bourne, Massachusetts, USA.
- Onset Computer Corporation. 2009. HOBOWare® Lite version 2.7.2. Bourne, Massachusetts, USA.
- O’Steen, S. 1998. Embryonic temperature influences juvenile temperature choice and growth rate in snapping turtles (*Chelydra serpentina*). *The Journal of Experimental Biology* 201:439–449.
- Packard, G. C., and M. J. Packard. 2004. To freeze or not to freeze: adaptations for overwintering by hatchlings of the North American painted turtle. *Journal of Experimental Biology* 207:2897–2906.
- Paitz, R. T., H. K. Harms, R. M. Bowden, and F. J. Janzen. 2007. Experience pays: offspring survival increases with female age. *Biology Letters* 3:44–46.

- Pieau, C. 1982. Modalities of the action of temperature on sexual differentiation in field-developing embryos of the European pond turtle *Emys orbicularis* (Emydidae). *Journal of Experimental Zoology* 220:353–360.
- Place, A. R., and V. A. Lance. 2004. The temperature-dependent sex determination drama. Pp. 99–110. In N. Valenzuela and V. A. Lance (Eds.), *Temperature-dependent Sex Determination in Vertebrates*. Smithsonian Institution Press., Washington D.C.
- Raynaud, A., and C. Pieau. 1985. Sexual differentiation as a function of incubation temperature. Pp. 264–300. In Gans, C. (Ed.), *Biology of the Reptilia Vol. 15*. John Wiley and Sons., New York.
- Rhen, T., and J. W. Lang. 2004. Phenotypic effects of incubation temperature in reptiles. Pp. 90–98. In N. Valenzuela and V. A. Lance (Eds.), *Temperature-dependent Sex Determination in Vertebrates*. Smithsonian Institution Press., Washington D.C.
- Rollinson, N., and R. J. Brooks. 2008. Sources and significance of among-individual reproductive variation in a northern population of painted turtles (*Chrysemys picta*). *Copeia* 2008:533–541.
- Schwarzkopf, L., and R. J. Brooks. 1985. Sex determination in northern painted turtles-effect of incubation at constant and fluctuating temperatures. *Canadian Journal of Zoology* 63:2543–2547.
- . 1987. Nest-site selection and offspring sex ratio in painted turtles, *Chrysemys picta*. *Copeia* 1987:53–61.
- Spetz, J. C. 2008. Diet, habitat use, and reproduction characteristics in an Ohio population of blanding's turtle (*Emydoidea blandingii*) in a lake Erie coastal plains marsh. Thesis (M.S.)-John Carroll University, University Heights, OH.
- SPSS Incorporated. 2008. *Statistical Package for the Social Sciences version 16.0*. Chicago, Illinois, USA.
- Tinkle, D. W., J. D. Congdon, and P. C. Rosen. 1981. Nesting frequency and success: implications for the demography of painted turtles. *Ecology* 62:1426–1432.
- Tucker, J. K., N. I. Filoramo, G. L. Paukstis, and F. J. Janzen. 1998. Response of red-eared slider, *Trachemys scripta elegans*, eggs to slightly differing water potentials. *Journal of Herpetology* 32:124–128.
- Tucker, J. K. 2000. Annual variation in hatchling size in the Red-Eared Slider Turtle (*Trachemys scripta elegans*). *Herpetologica* 56:8–13.

- Valenzuela, N. 2004. Introduction. Pp. 1–4. *In* N. Valenzuela and V. A. Lance (Eds.), *Temperature-dependent Sex Determination in Vertebrates*. Smithsonian Institution Press., Washington D.C.
- Vogt, R. C., and J. J. Bull. 1982. Temperature controlled sex-determination in turtles: Ecological and behavioral aspects. *Herpetologica* 38:156–164.
- . 1984. Ecology of hatchling sex ratio in map turtles. *Ecology* 65:582–587.
- Weisrock D. W., and F. J. Janzen. 1999. Thermal and fitness-related consequences of nest location in painted turtles (*Chrysemys picta*). *Functional Ecology* 13:94–101.
- Wilson, D. S. 1998. Nest-site selection: microhabitat variation and its effects on the survival of turtle embryos. *Ecology* 79:1884–1892.
- Yntema, C. L. 1981. Characteristics of gonads and oviducts in hatchlings and young of *Chelydra serpentina* resulting from three incubation temperatures. *Journal of Morphology* 167:297–304.

**Table 1.** Breakdown of total field research hours conducted at The Rookery and The West Woods, Geauga County, Ohio during the summer of 2009.

<b>Researchers</b>	<b>Total field research hours</b>	<b>Nest searching hours</b>	<b>*Trapping/other hours</b>
<b>Total</b>	845.8	637.5 (median = 7.5)	202.55

\* Trapping/other hours: all trapping hours plus downloading data-loggers, estimating canopy-cover and measuring/recording the nest variables. Note: there were 44 days of trapping (May 8–June 5; June 29–July 2; July 14–July 31), and traps were set for an average 23.24 hours for each event).

**Table 2A:** Number of *Chrysemys picta marginata* and *Chelydra serpentina* hoop-net trapped at The Rookery and The West Woods, Geauga County, Ohio, USA.

<b>The Rookery</b>	<b>Male</b>	<b>Female</b>	<b>Total</b>
<i>C. picta</i> Capture	20	11	31
<i>C. picta</i> Recapture	3	1	4
<i>C. serpentina</i> Capture	11	8	19
<i>C. serpentina</i> Recapture	3	3	6
<b>The West Woods</b>			
<i>C. picta</i> Capture	2	1	3
<i>C. picta</i> Recapture	0	1	1
<i>C. serpentina</i> Capture	0	0	0
<i>C. serpentina</i> Recapture	0	0	0

**Table 2B:** Number of *Chrysemys picta marginata* and *Chelydra serpentina* hand-caught at The Rookery and The West Woods, Geauga County, Ohio, USA.

<b>The Rookery</b>	<b>Male</b>	<b>Female</b>	<b>Total</b>
<i>C. picta</i> Capture	2	33	35
<i>C. picta</i> Recapture	0	10	10
<i>C. serpentina</i> Capture	0	3	3
<i>C. serpentina</i> Recapture	0	0	0
<b>The West Woods</b>			
<i>C. picta</i> Capture	0	9	9
<i>C. picta</i> Recapture	0	2	2
<i>C. serpentina</i> Capture	0	1	1
<i>C. serpentina</i> Recapture	0	0	0

**Table 3.** Standardized Beta coefficients for a multiple linear regression of the relationship between 12 nest attributes and their respective mean temperatures experienced during the critical thermosensitive period of natural incubation of *Chrysemys picta marginata* (i.e., the middle 25 days of a 75 day incubation period) (Mean Nest Temperature =  $-0.6080.932 + 0.000(\text{Lay Date}) - 0.087(\text{Clutch Size}) + 2.980(\text{Slope}) - 0.064(\text{Aspect}) + 0.000(\text{D Water}) - 0.009(\text{D Forbs}) - 0.007(\text{F Grasses}) - 0.000(\text{D Trees/Shrubs}) + 0.013(\text{NVS}) - 0.007(\text{EVS}) - 0.009(\text{SVS}) - 0.008(\text{WVS})$ ).

<b>Nest attributes</b>	<b>Std. <math>\beta</math> coefficients</b>	<b><i>t</i></b>	<b><i>P</i></b>
Lay date	0.351	2.048	0.057
Clutch size	-0.150	-0.725	0.479
Slope (cm)	0.196	1.264	0.244
Aspect	-0.143	-0.778	0.448
*D Water (cm)	0.210	1.031	0.318
*D Forbs (cm)	-0.135	-0.879	0.393
*D Grasses (cm)	-0.127	-0.648	0.526
*D Trees/Shrubs (cm)	0.027	0.113	0.911
**NCC (%)	0.504	2.327	0.033
**ECC (%)	-0.294	-1.175	0.257
**SCC (%)	-0.309	-1.355	0.194
**WCC (%)	-0.284	-1.140	0.271

\*Distances to the closest water source (single measurement), forbs, grasses, and trees/shrubs (3 replicate measurements averaged).

\*\*Percentages of canopy cover (CC) in each cardinal direction.

**Table 4.** Standardized Beta coefficients for a weight-least-squares linear regression of the relationship between mean nest temperature and clutch size and the subsequent percentage of *C. p. marginata* males in the nests (Percent Males = 2.425 – 0.040(Mean Temperature) – 0.088(Clutch Size)).

<b>Nest attributes</b>	<b>Std. <math>\beta</math> coefficients</b>	<b><i>t</i></b>	<b><i>P</i></b>
Mean Temperature	-0.162	-0.894	0.382
Clutch size	-0.657	-3.624	0.002

**Figure 1.** A hypothetical subset of a temperature trace corresponding to sex determination in TSD turtles. The solid line indicates the hypothetical incubation temperature trace that would be recorded within a nest, whereas the dotted gray-box depicts the hypothetical thermosensitive period (TSP), which determines the sex of an embryo that exhibits TSD. The dotted gray-line illustrates the pivotal temperature ( $T_{piv}$ ) at which a 1:1 secondary sex ratio would be produced in this particular TSD turtle.

**Figure 2.** Patterns of TSD (TSD Ia (A), TSD Ib (B), TSD II (C) characterized under constant laboratory incubation temperatures. The dotted gray-box shown in TSD Ib illustrates the transitional range of temperatures that would produce both male and female offspring (same would be seen in TSD Ia). The arrows depicted in TSD Ia and TSD II illustrates the  $T_{piv}$  (i.e., corresponding to a 1:1 secondary sex ratio (small dotted line); Valenzuela, 2004).

**Figure 3.** Geographic distribution of the Painted Turtle (*Chrysemys picta*) including the 4 subspecies: *C. p. marginata* (Midland); *C. p. dorsalis* (Southern); *C. p. bellii* (Western); and *C. p. picta* (Eastern) (Conant and Collins, 1998).

**Figure 4.** Map of Geauga Park District illustrating the location of The Rookery (41°29'59.41"N, 81°17'32.49"W), and The West Woods (41°27'27.82"N, 81°18'13.50"W) where this research was performed (Gauga Park District, 2008).

**Figure 5.** Temperature trace from nest C.p.\_31 at The Rookery. The red box corresponds to the middle third (i.e., 25 days) of incubation, and the 28.5°C  $T_{piv}$  is accounted for by the solid black line (HOBOWare® Lite V2.7.2; Onset Computer Corporation, 2009).

**Figure 6.** Location of 6 monitored (red boxes and nest numbers) and 9 predated (yellow triangles) nests of *Chrysemys picta marginata* at The West Woods, Geauga County, Ohio (41°27'27.82"N, 81° 18'13.50"W).

**Figure 7.** Location of 30 nests of *Chrysemys picta marginata* and 2 nests of *Chelydra serpentina* (C.s.\_1 and C.s.\_2) at The Rookery, Geauga County, Ohio (41°29'59.41"N, 81°17'32.49"W).

**Figure 8.** Mean nest temperatures for the 29 nests of *Chrysemys picta marginata* at The Rookery (Note: nests C.p.\_13, 20, 24, 27, 28, 29, 31, 32, 34, and 36 either had dead eggs or individuals that did not hatch fully; see text).

**Figure 9.** Relationship between the amount of canopy cover over nests of *Chrysemys picta marginata* at The Rookery from all 4 cardinal directions (N, E, S, W) and the percentage of males that were produced from each nest (N = 29).

**Figure 10.** Relationship between adult female carapace length and hatchling carapace length of *Chrysemys picta marginata* at The Rookery (N = 70).

**Figure 11.** Relationship between mean nest temperature and hatchling mass (A) and hatchling carapace and plastron length (B) of *Chrysemys picta marginata* at The Rookery (N = 80; gray triangles = plastron length; black squares = carapace length).

**Figure 12.** Relationship between the amount of canopy cover over nests of *Chrysemys picta marginata* at The Rookery from all 4 cardinal directions (N, E, S, W) and hatchling carapace length as a proxy of size (N = 80).

**Figure 13.** Relationship between the amount of canopy cover over nests of *Chrysemys picta marginata* at The Rookery from all 4 cardinal directions (N, E, S, W) and hatchling sprint speed (N = 80).

**Figure 14.** Relationship between the amount of canopy cover over nests of *Chrysemys picta marginata* at The Rookery from all 4 cardinal directions (N, E, S, W) and hatchling righting time (N = 80).

**Figure 15.** Relationship between mean nest temperature and hatchling sprint speed (A) and hatchling righting time (B) of *Chrysemys picta marginata* at The Rookery (N = 80).

**Figure 16.** Relationship between hatchling sex and carapace length of *Chrysemys picta marginata* at The Rookery (N = 80; male SE  $\pm$  0.202; female SE  $\pm$  0.246).

**Figure 17.** Relationship between male and female sprint speed of *Chrysemys picta marginata* at The Rookery (N = 80; male SE  $\pm$  0.117; female SE  $\pm$  0.632).

**Figure 18.** Relationship between male and female righting time of *Chrysemys picta marginata* at The Rookery (N = 80; male SE  $\pm$  2.686; female SE  $\pm$  8.663).

**Figure 19.** Relationship between sprint speed and hatchling mass (A), and sprint speed and hatchling length (carapace and plastron length) (B) of *Chrysemys picta marginata* at The Rookery (N = 80; gray triangles = plastron length; black squares = carapace length).

**Figure 20.** Relationship between righting time and hatchling mass (A), and righting time and hatchling length (carapace and plastron length) (B) of *Chrysemys picta marginata* at The Rookery (N = 80; gray triangles = plastron length; black squares = carapace length).

**Figure 1**

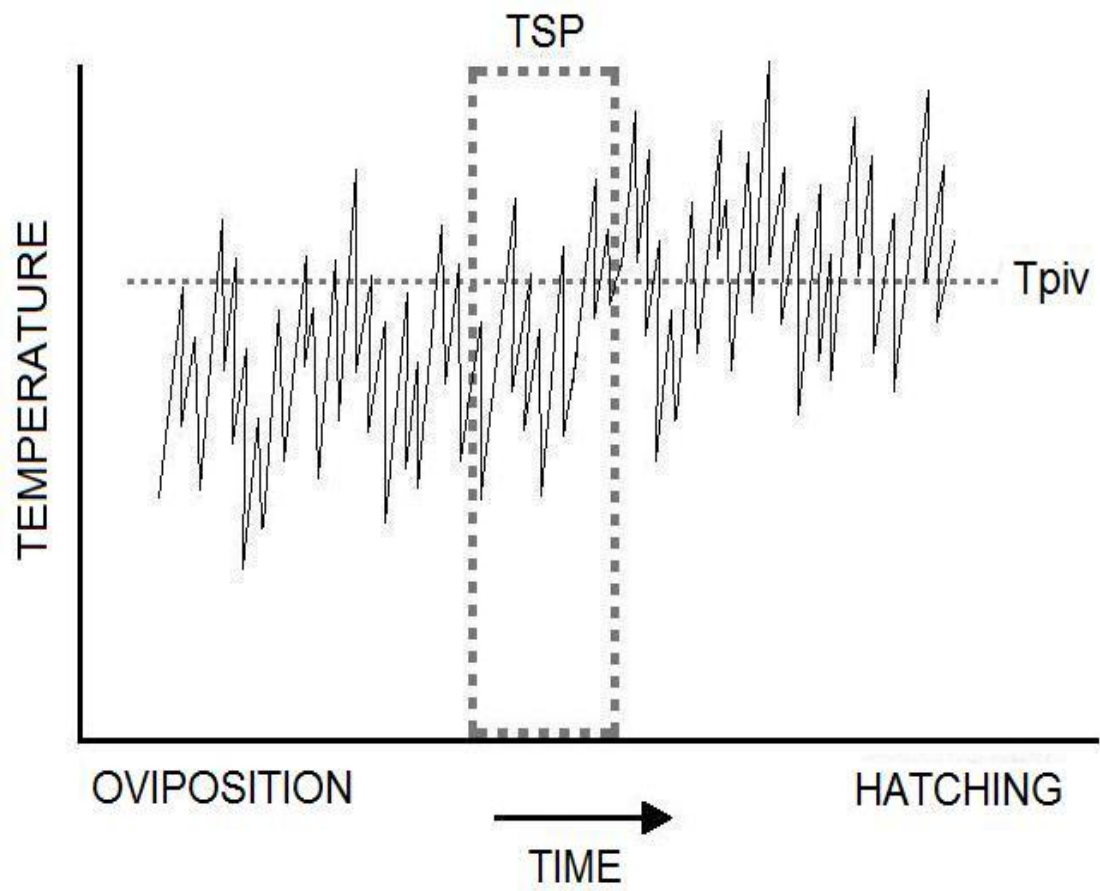


Figure 2A

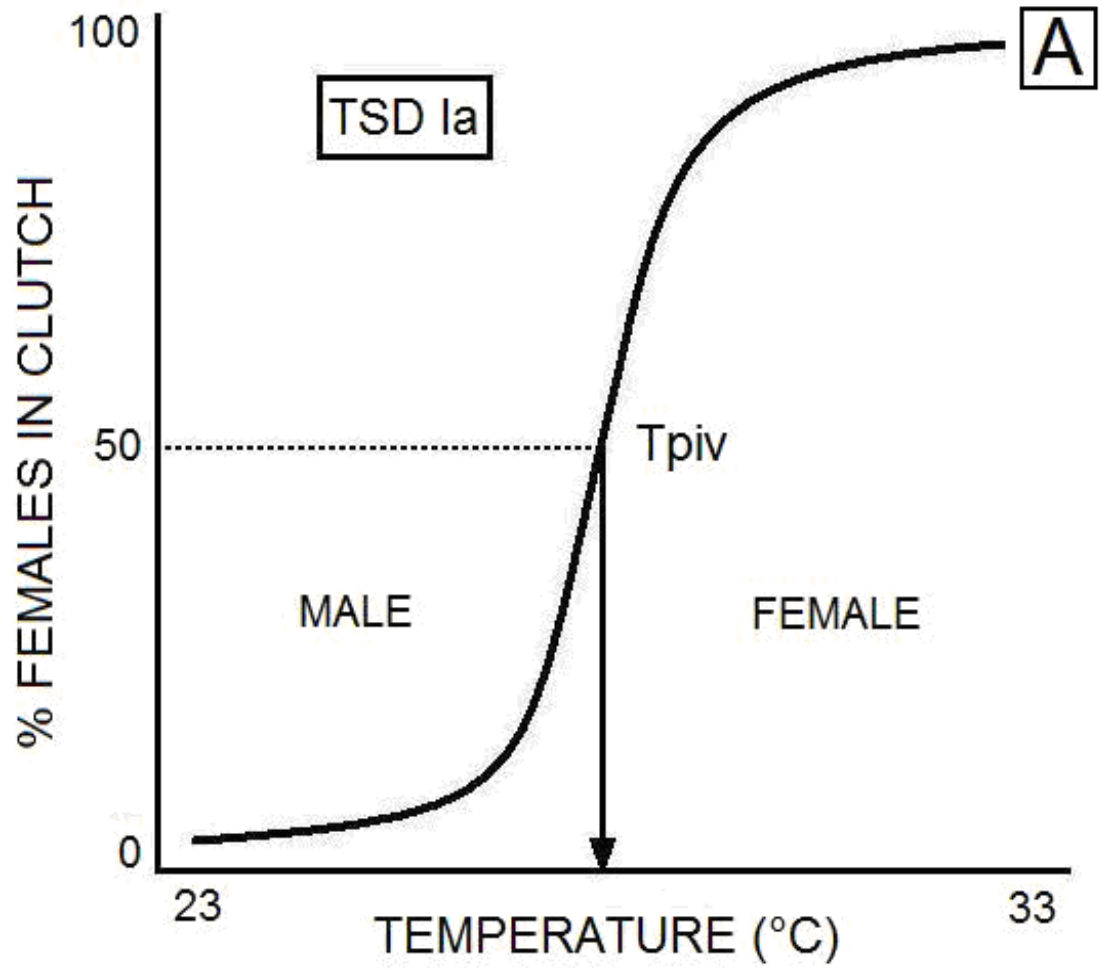
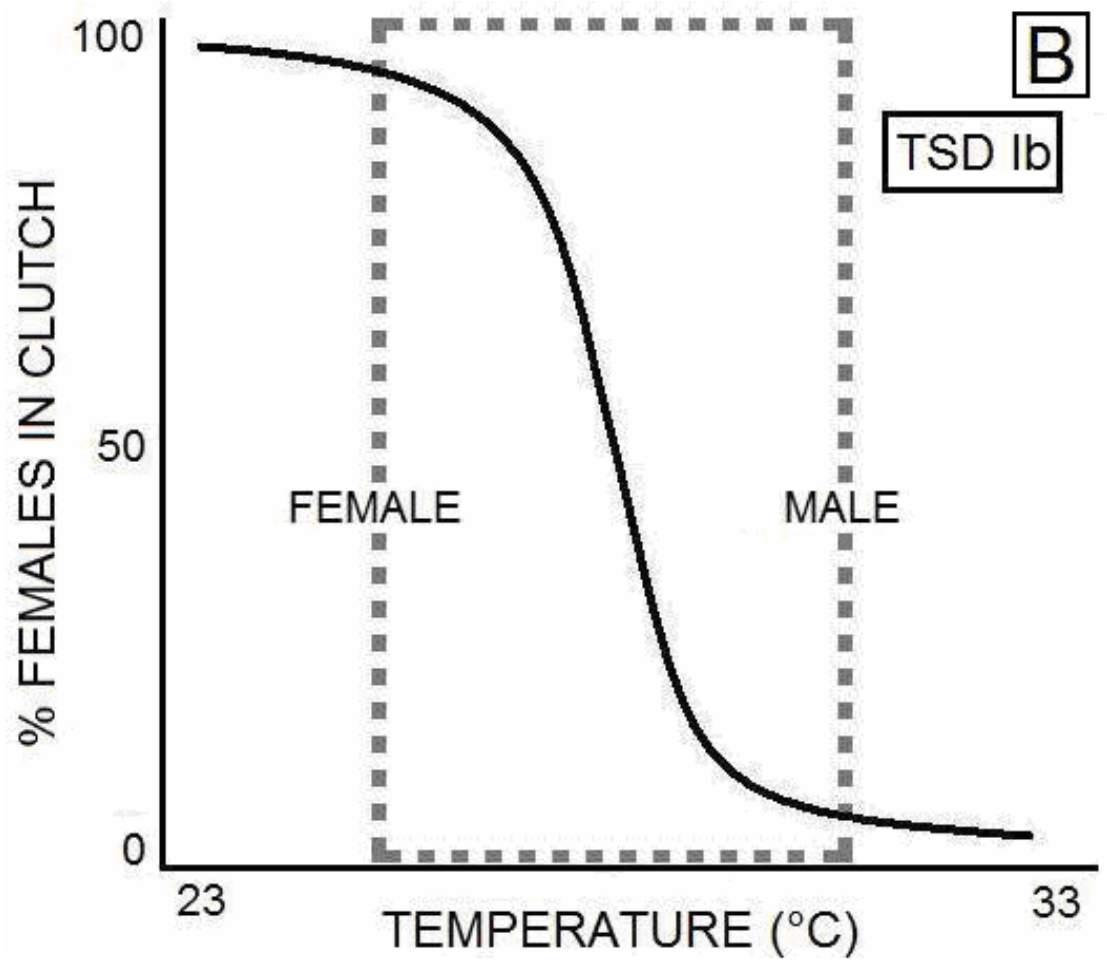


Figure 2B



**Figure 2C**

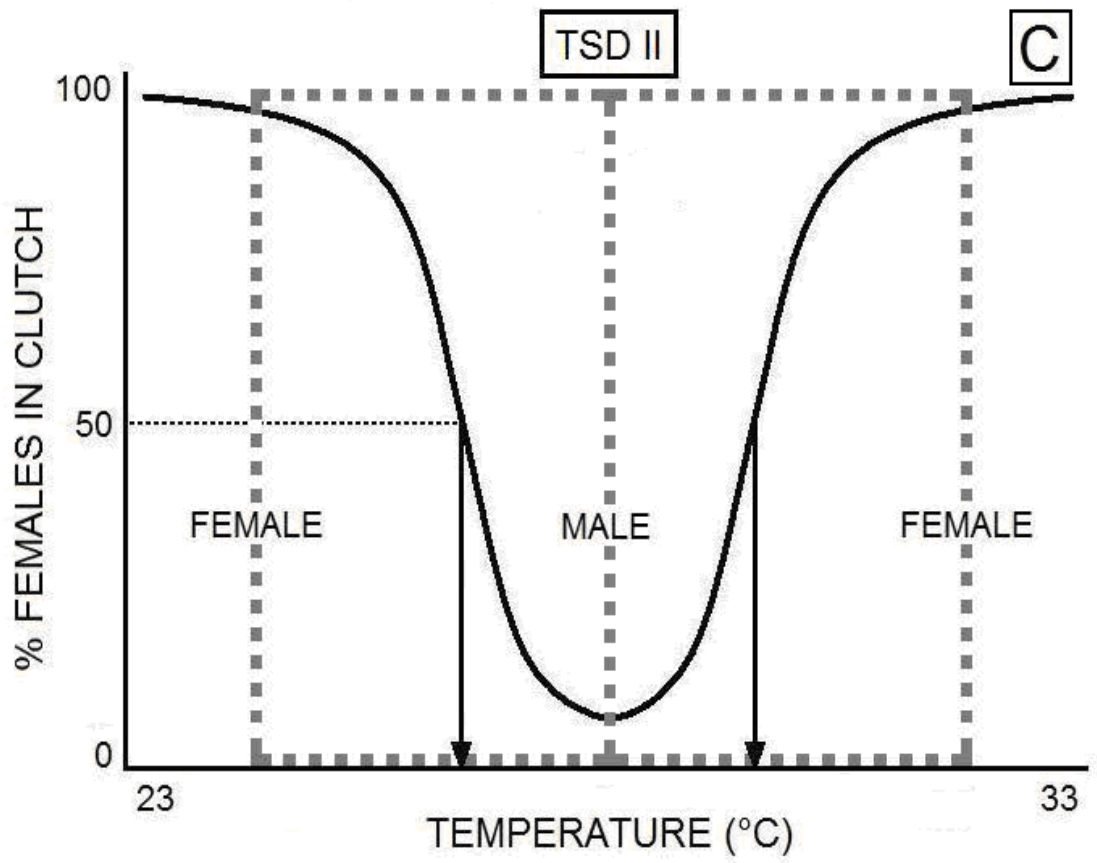
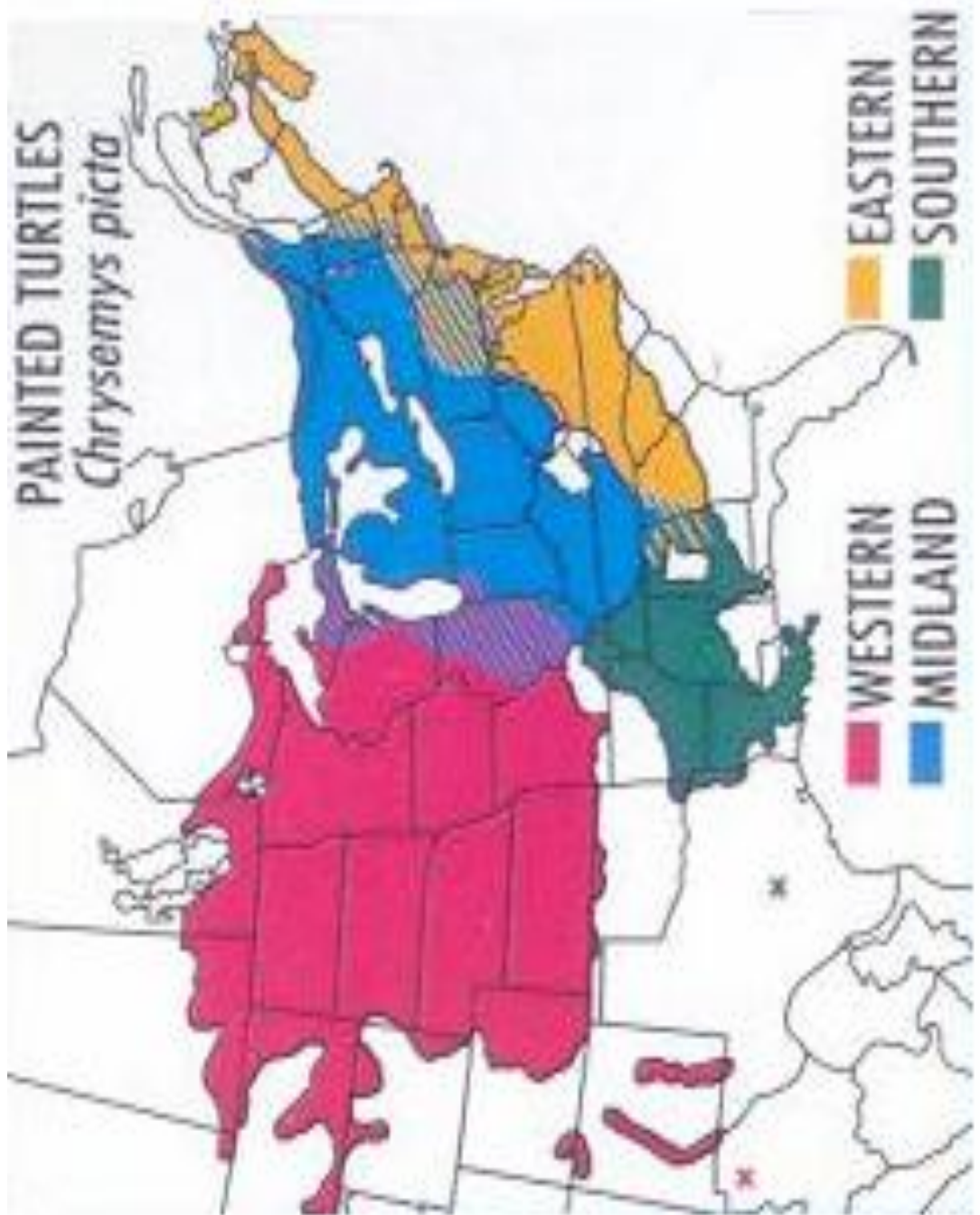
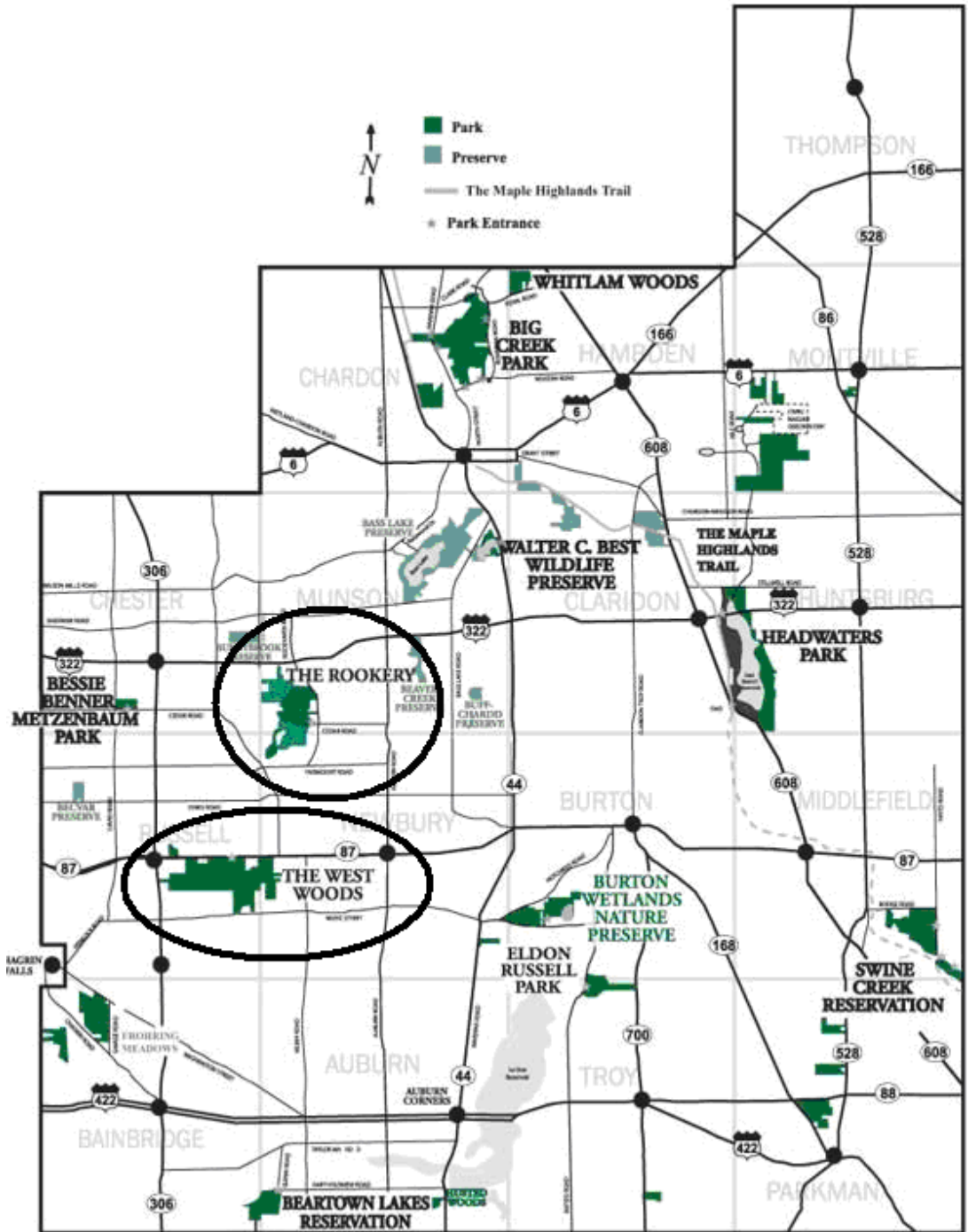


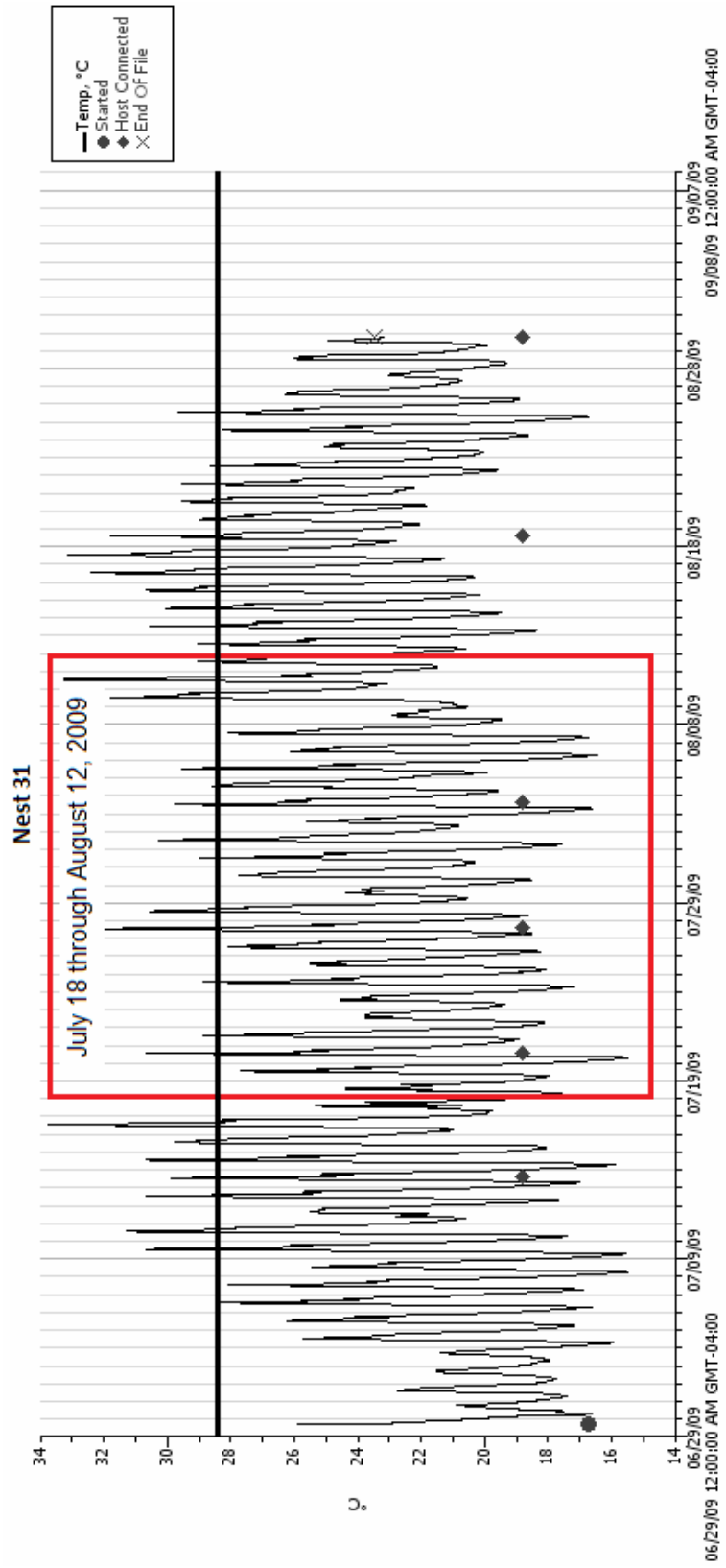
Figure 3



**Figure 4**



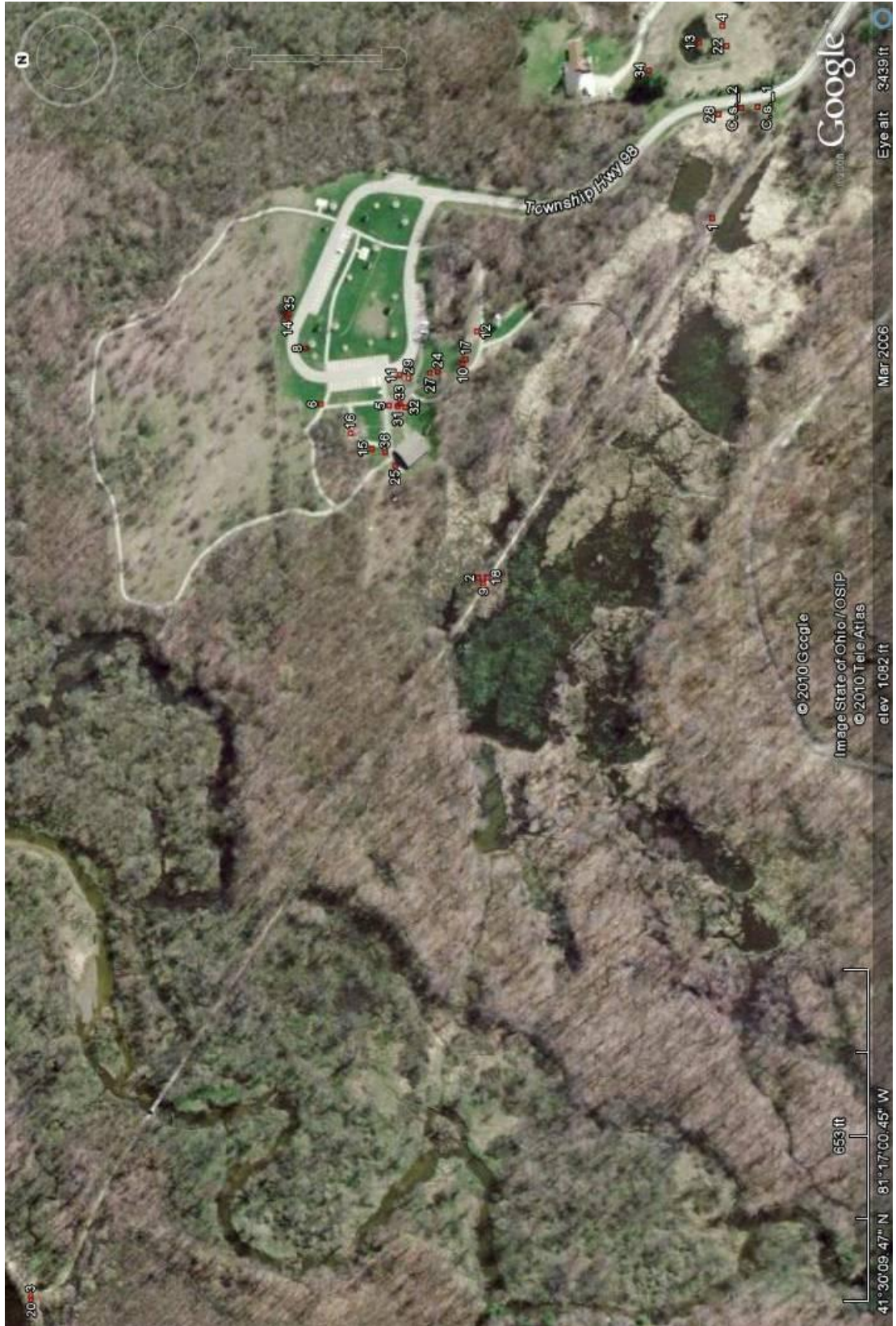
**Figure 5**

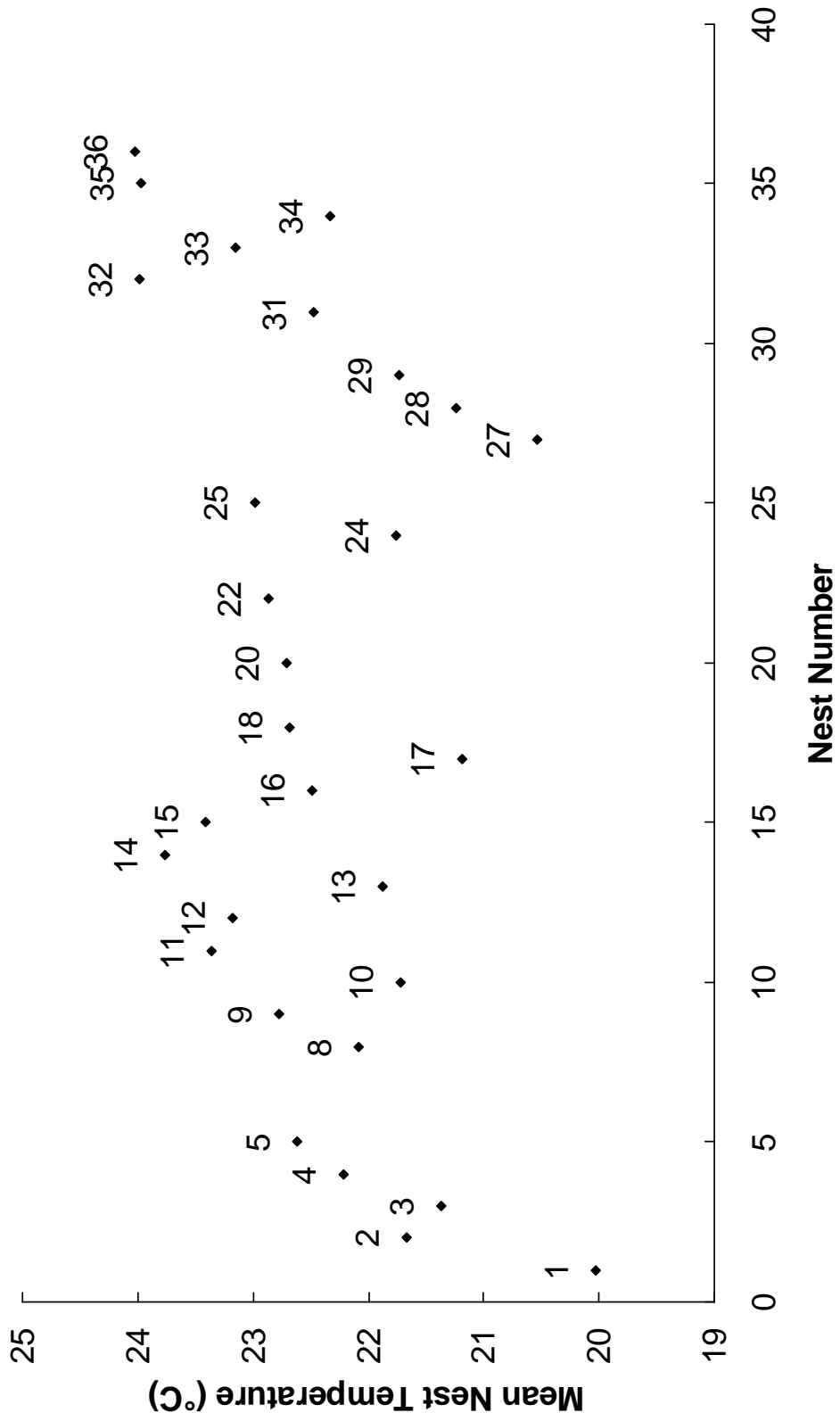


**Figure 6**



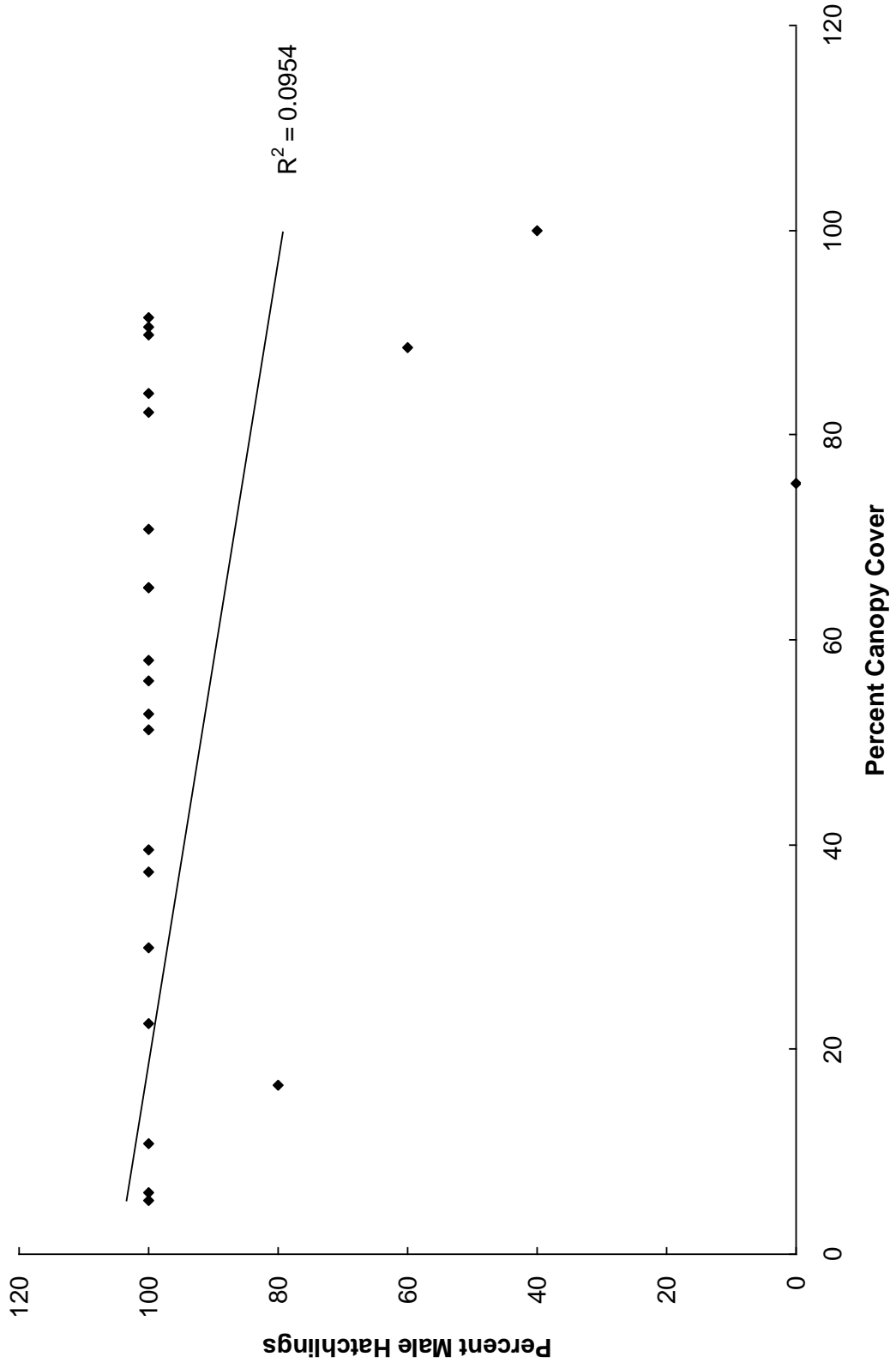
**Figure 7**





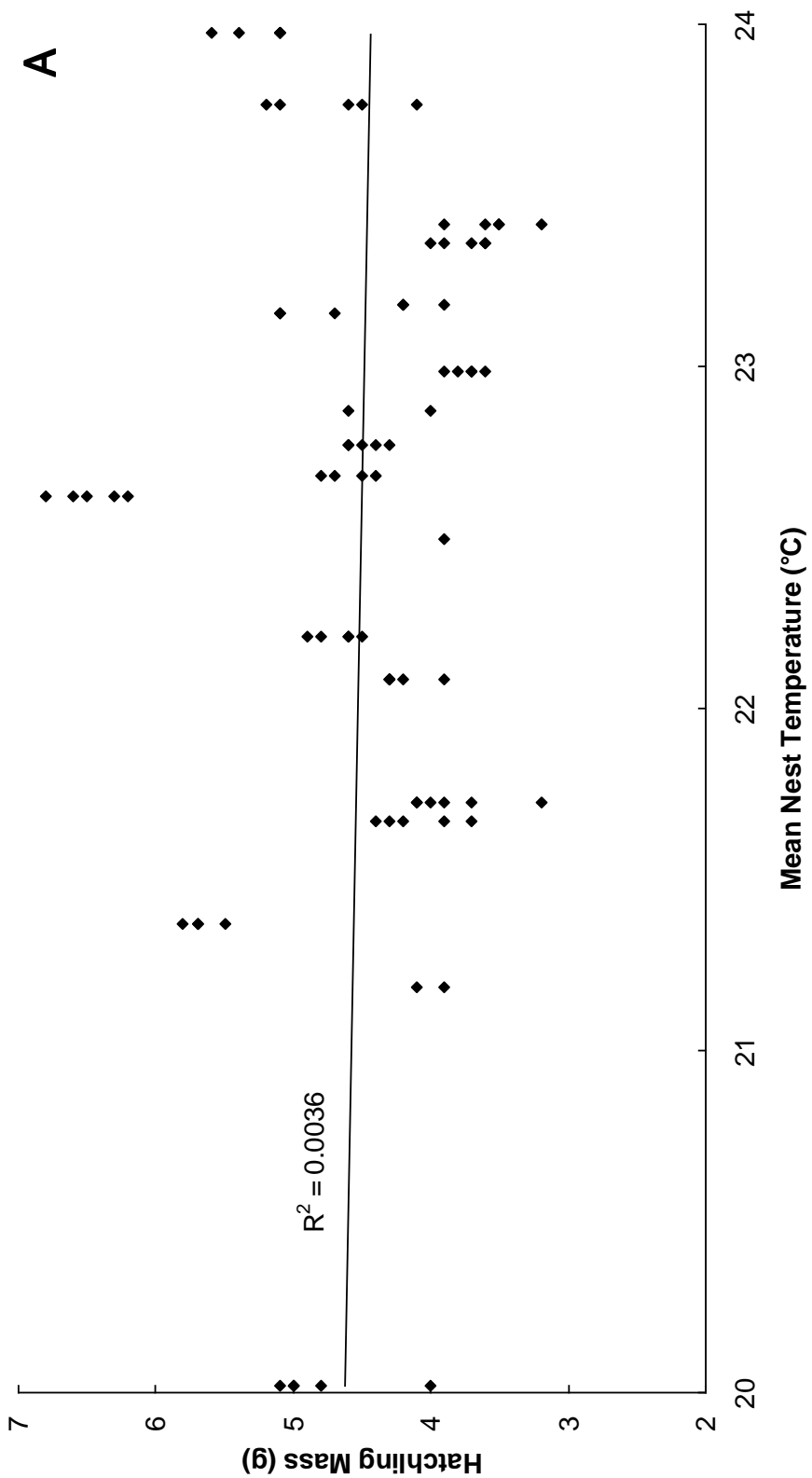
**Figure 8**

**Figure 9**

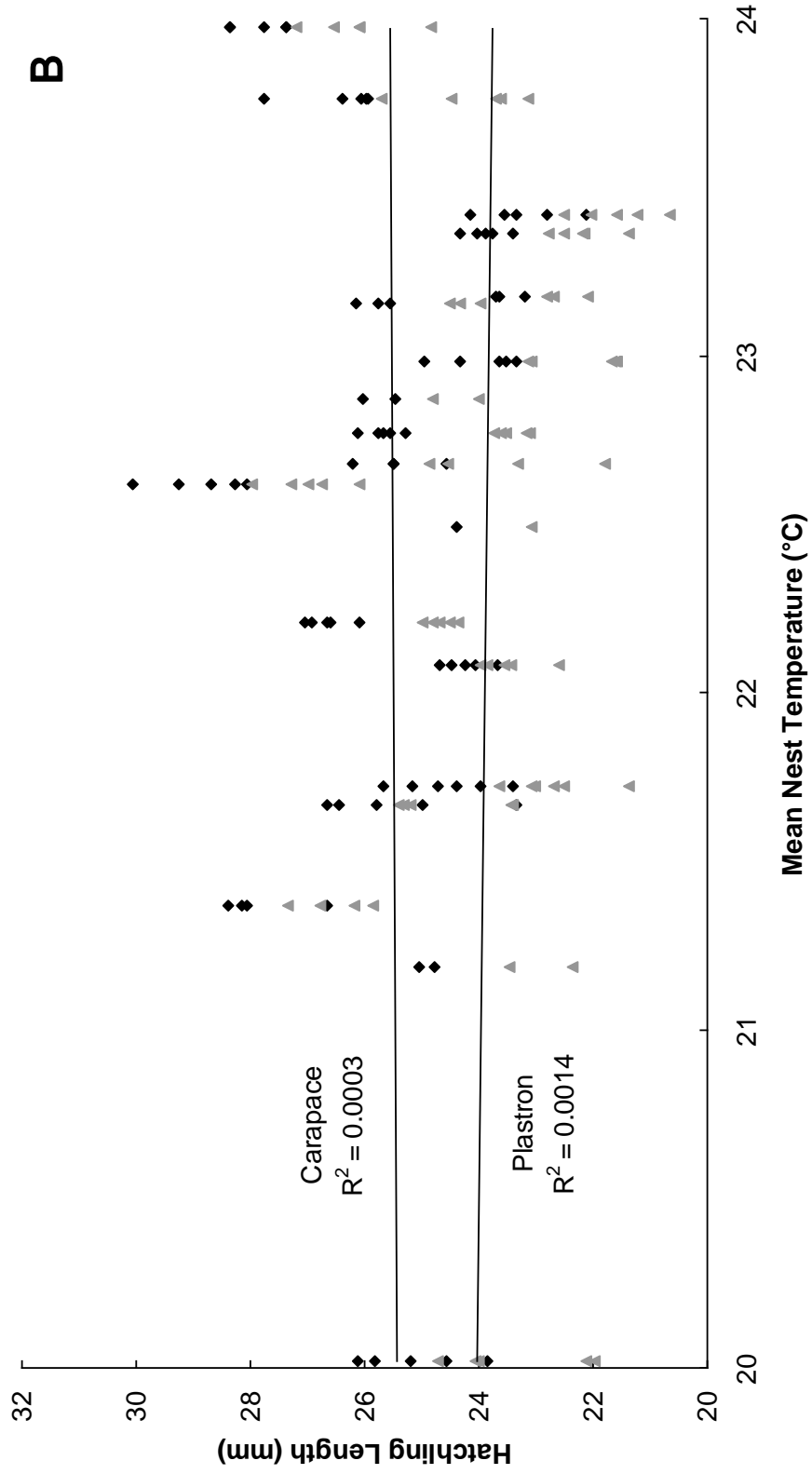




**Figure 11A**

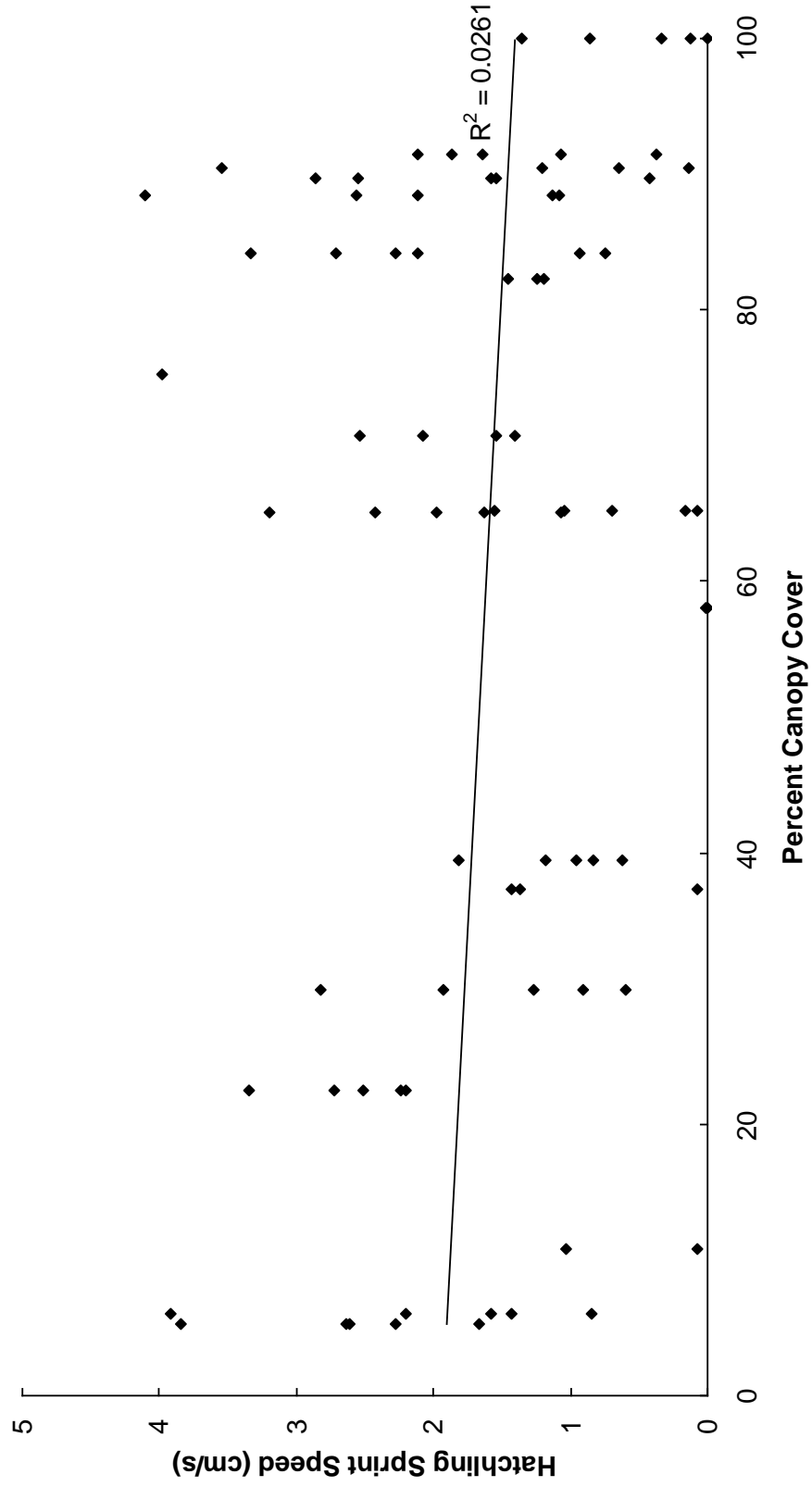


**Figure 11B**

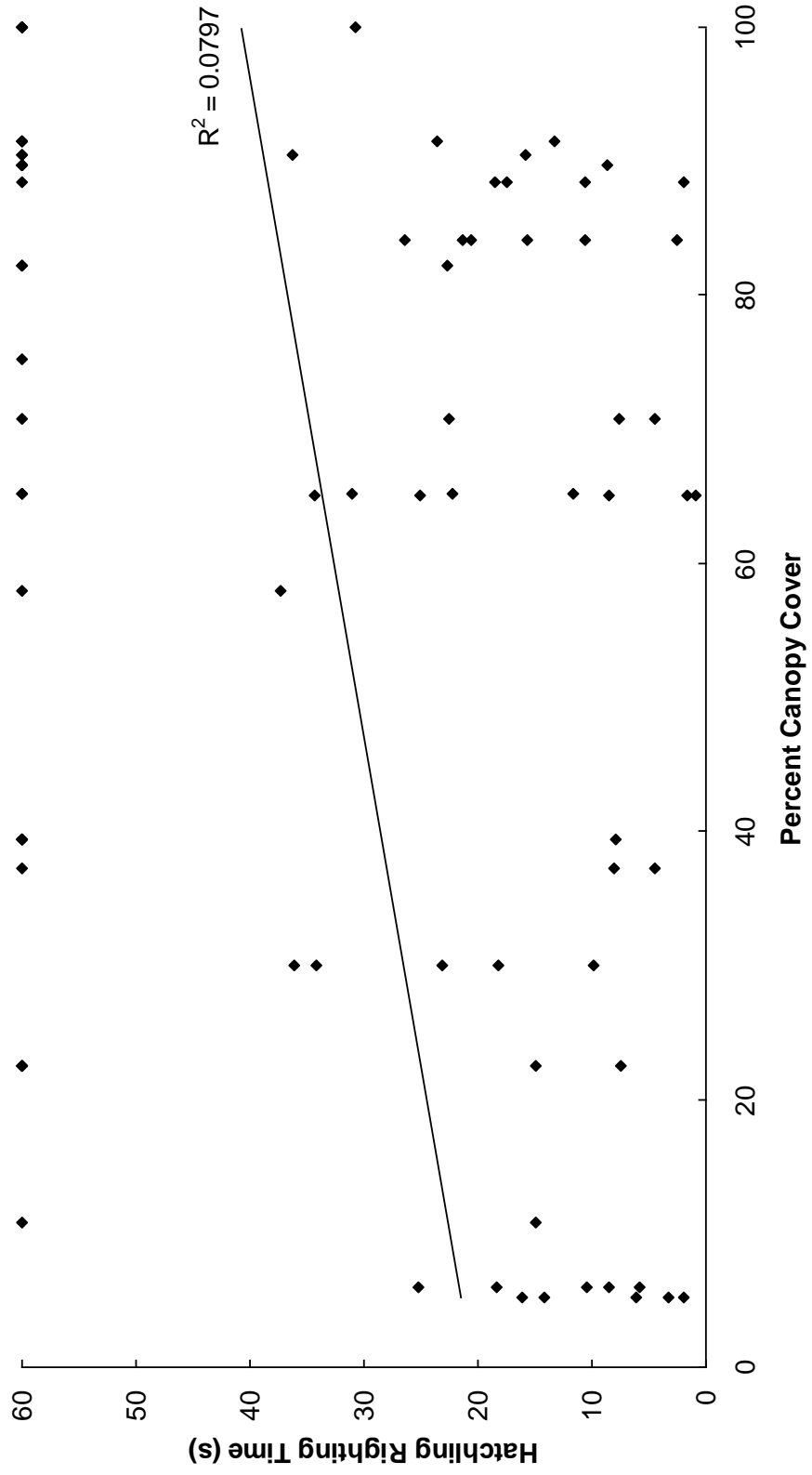




**Figure 13**

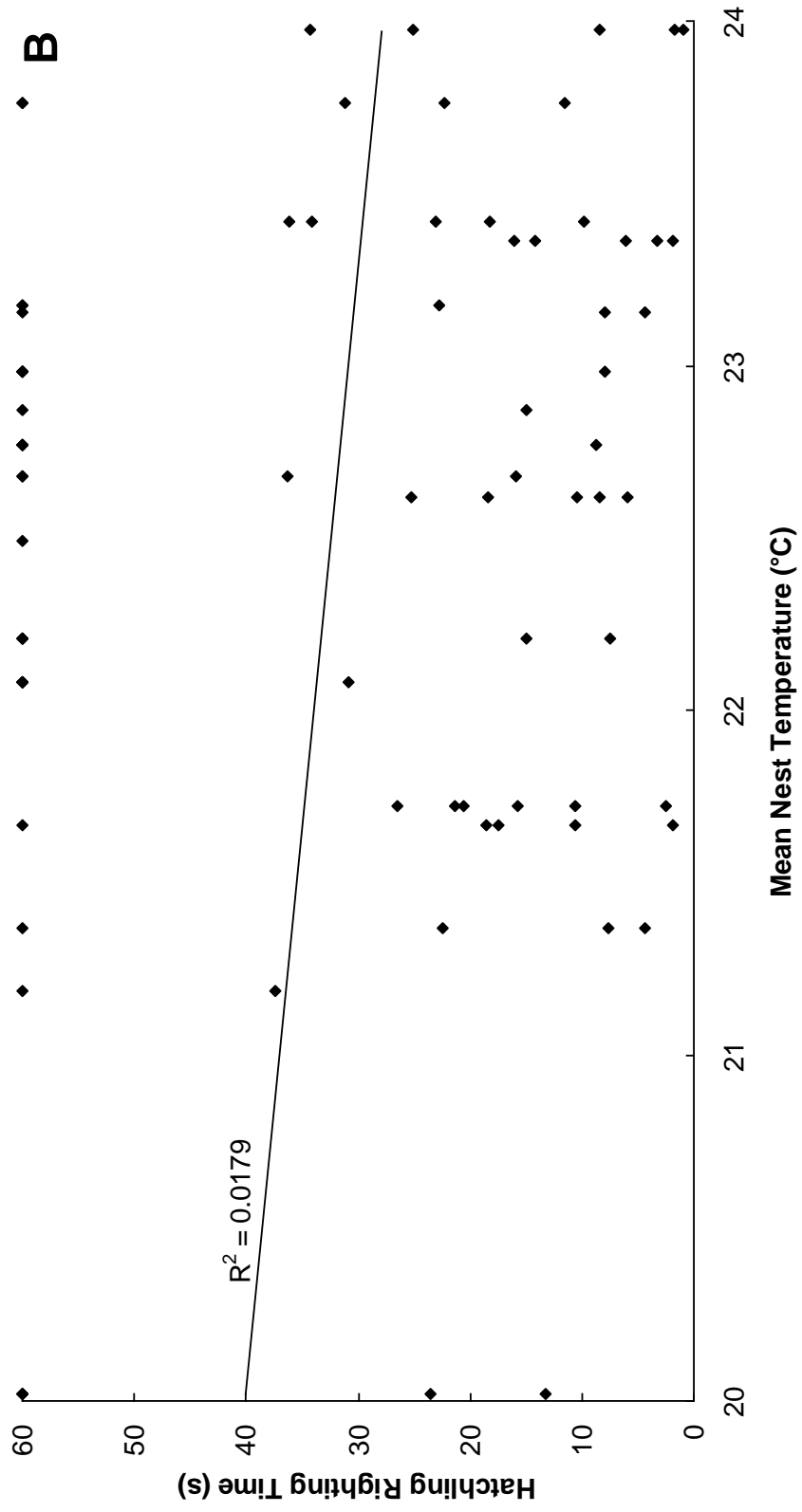


**Figure 14**

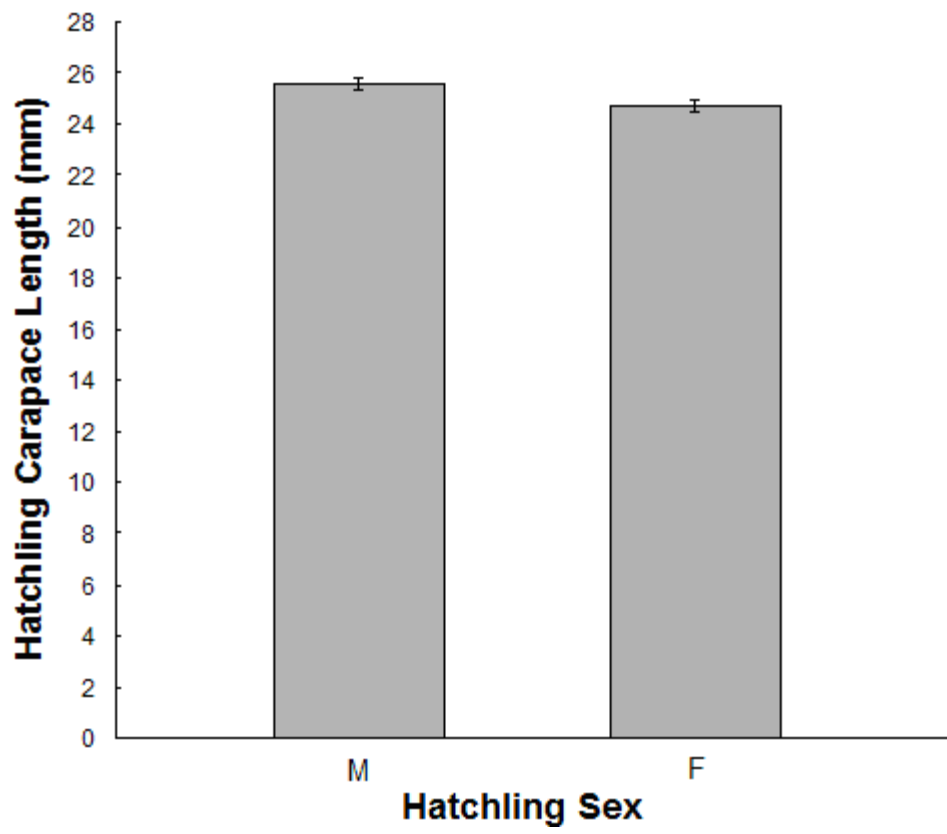




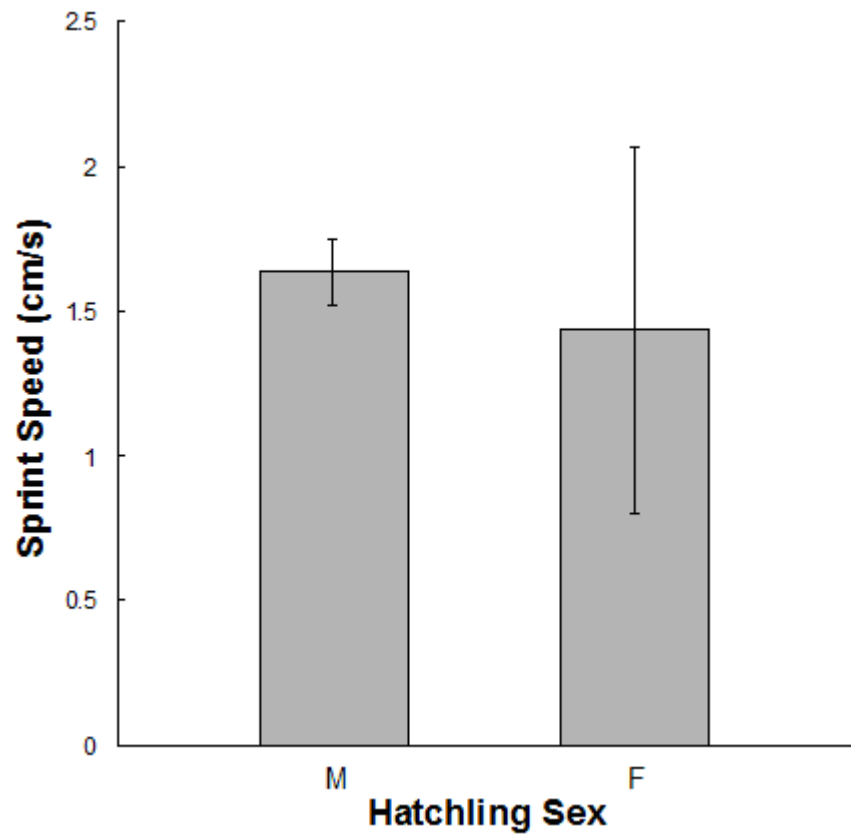
**Figure 15B**



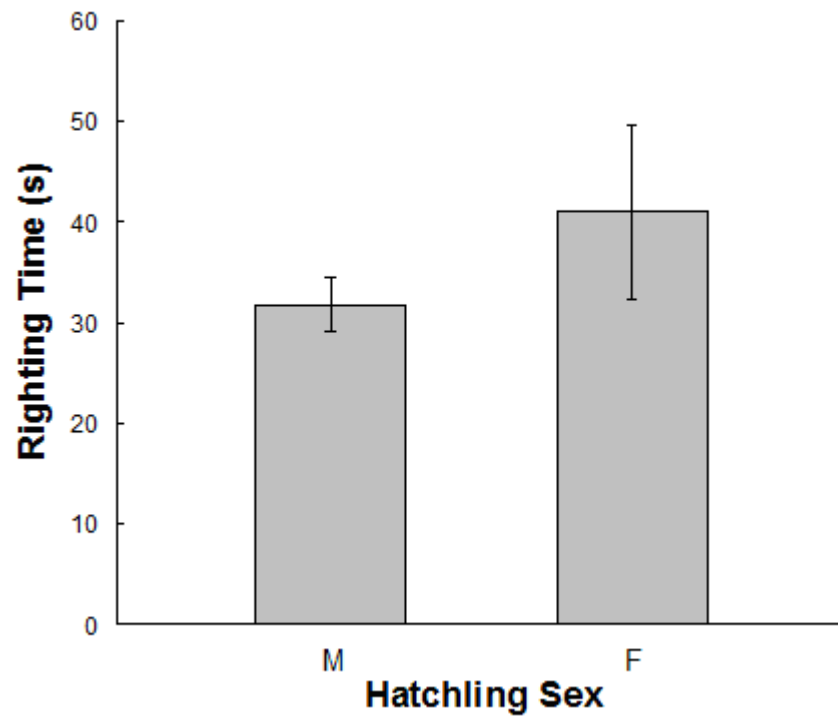
**Figure 16**



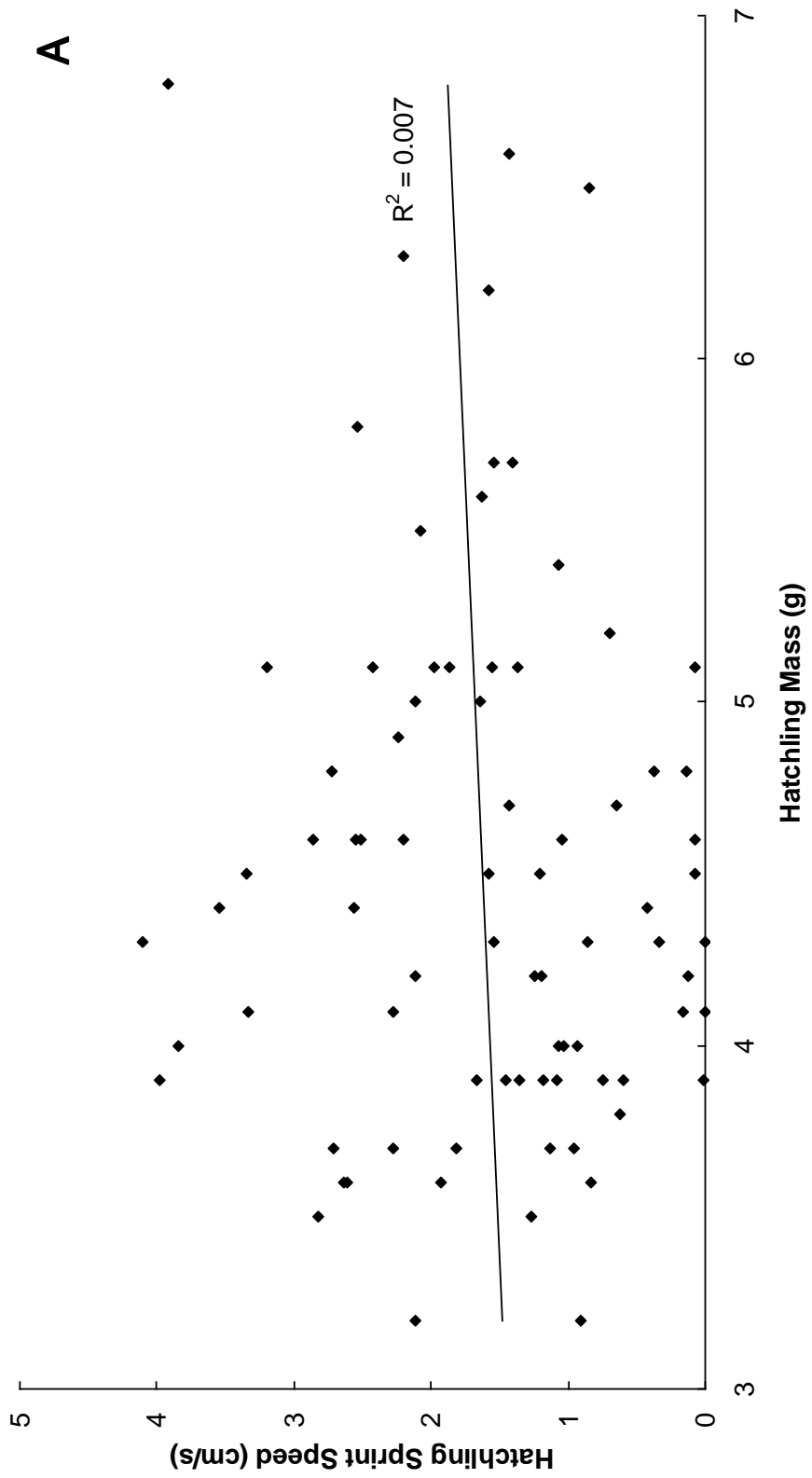
**Figure 17**



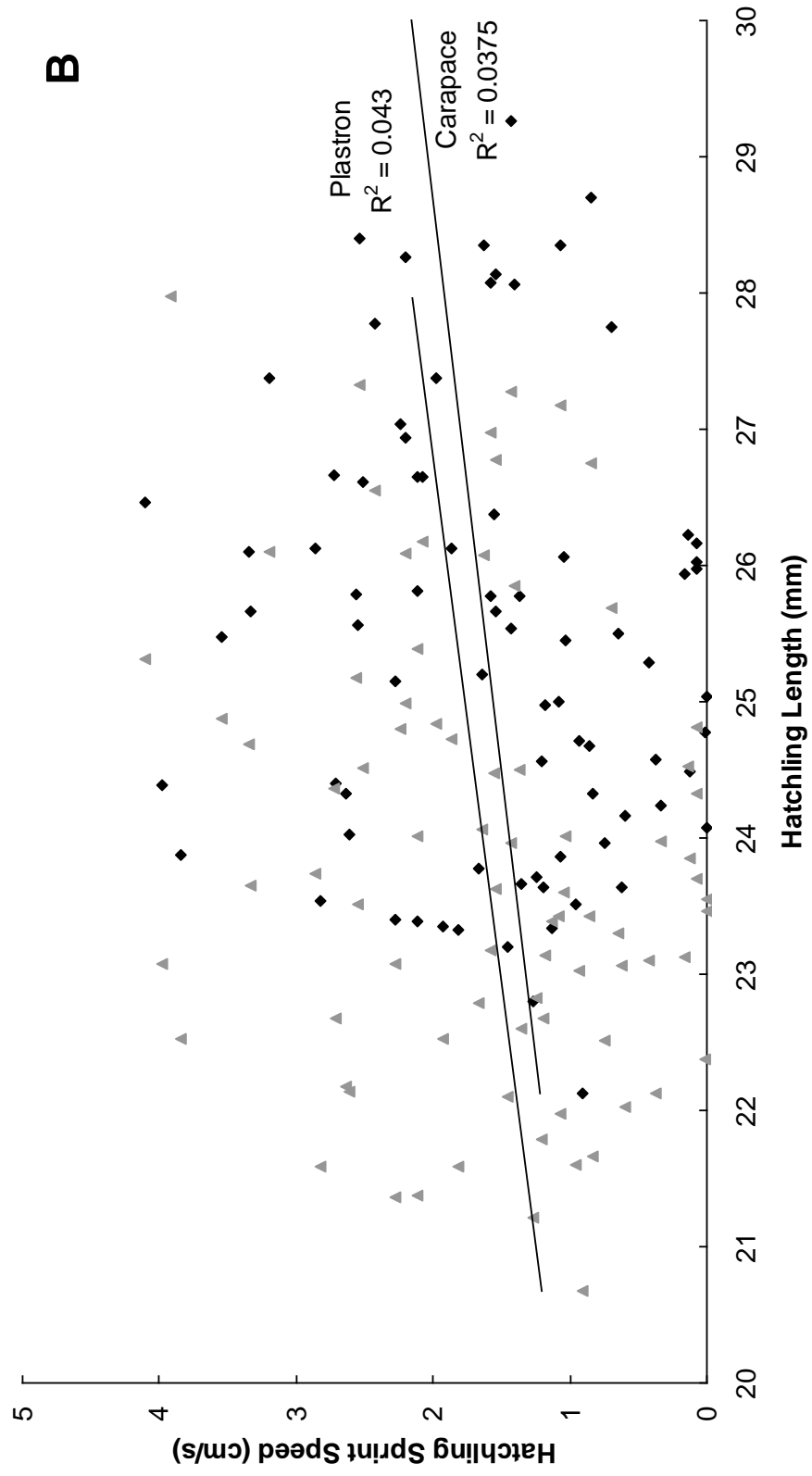
**Figure 18**



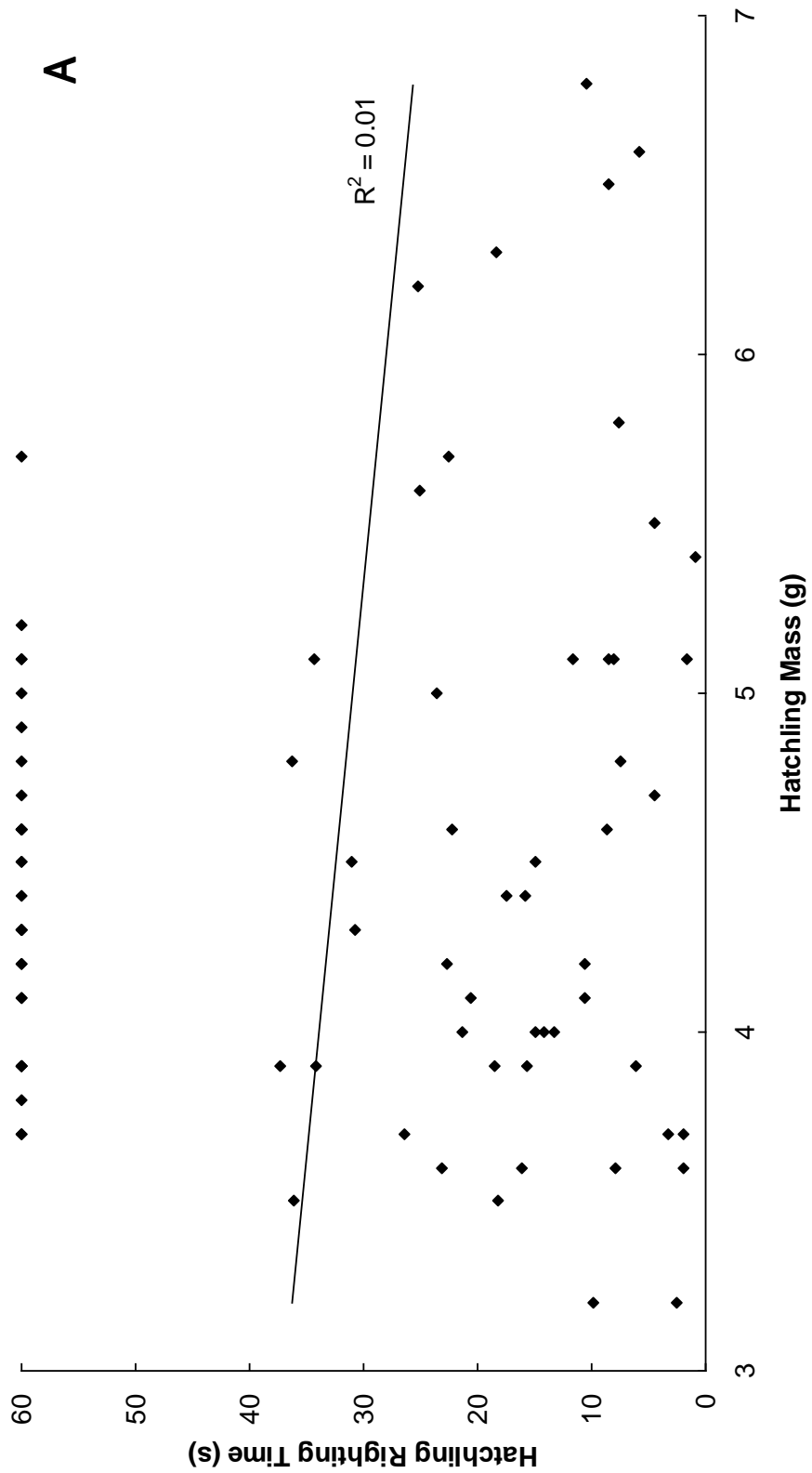
**Figure 19A**



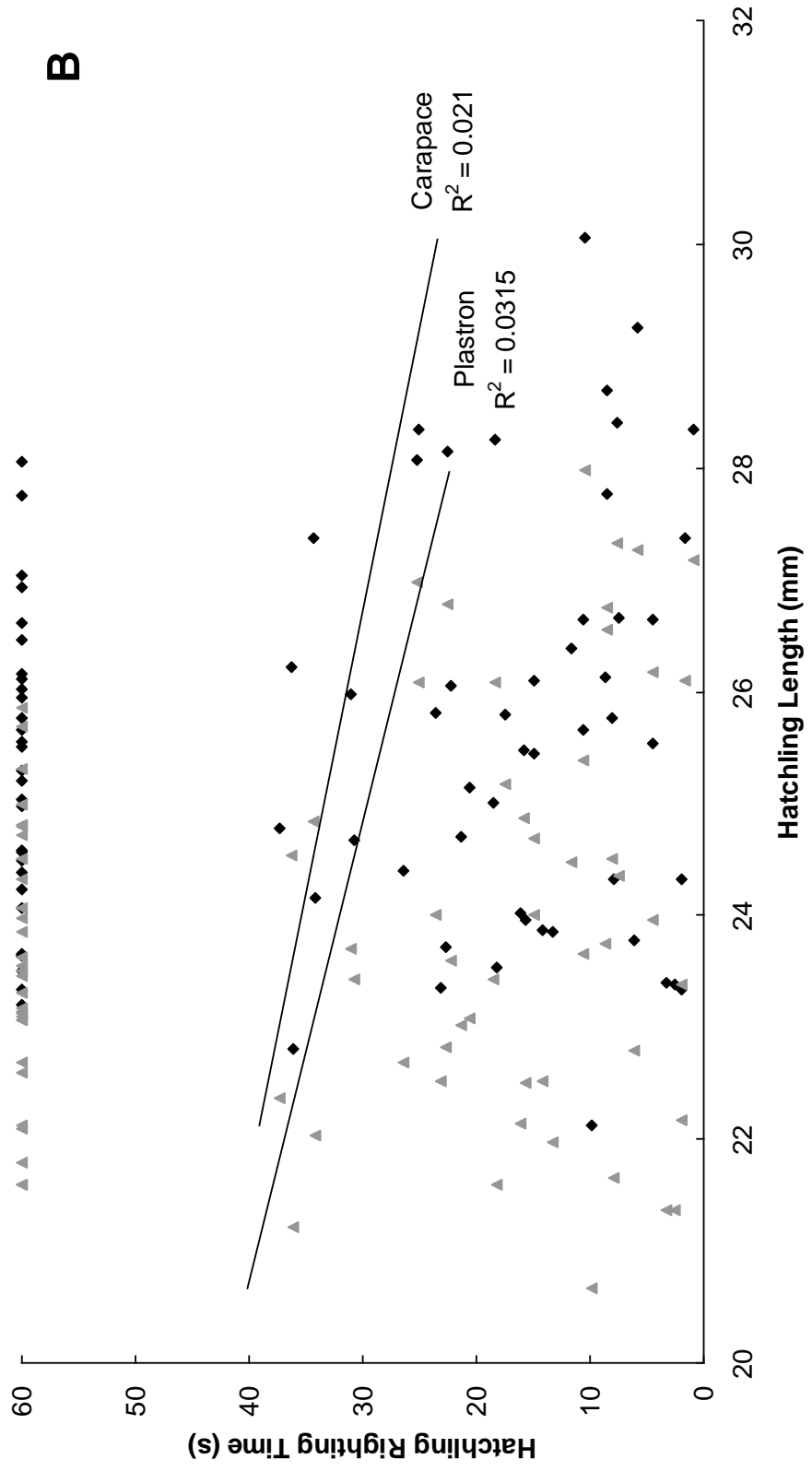
**Figure 19B**



**Figure 20A**



**Figure 20B**



**Appendix a:** Taxonomic distribution of Chelonia known to exhibit temperature-dependent sex determination.

	Taxa	TSD Type
SUBORDER: Cryptodira		
FAMILY: Emydidae		
SUBFAMILY: Emydinae		
	<i>Chrysemys picta</i>	TSD Ia
	<i>Clemmys guttata</i>	TSD Ia <sup>c</sup>
	<i>Deirochelys reticularia</i>	TSD Ia
	<i>Emydoidea blandingi</i>	TSD Ia
	<i>Emys orbicularis</i>	TSD <sup>amb</sup>
	<i>Graptemys barbouri</i>	TSD <sup>amb</sup>
	<i>G. geographica</i>	TSD Ia
	<i>G. kohna</i>	TSD <sup>amb</sup>
	<i>G. nigrinoda</i>	TSD <sup>amb</sup>
	<i>G. ouachitensis</i>	TSD <sup>amb</sup>
	<i>G. pseudogeographica</i>	TSD Ia
	<i>G. pulchra</i>	TSD <sup>amb</sup>
	<i>Malaclemys terrapin</i>	TSD <sup>amb</sup>
	<i>Pseudemys concinna</i>	TSD Ia
	<i>P. floridana</i>	TSD Ia
	<i>P. nelsoni</i>	TSD Ia
	<i>P. texana</i>	TSD Ia
	<i>Terrapene Carolina</i>	TSD <sup>amb</sup>
	<i>T. ornate</i>	TSD Ia
	<i>Trachemys decorate</i>	TSD Ia
	<i>T. scripta</i>	TSD Ia
SUBFAMILY: Batagurinae		
	<i>Chinemys nigricans</i>	TSD Ia <sup>c</sup>
	<i>C. reevesi</i>	TSD <sup>amb</sup>
	<i>Mauremys annamansis</i>	TSD Ia
	<i>M. mutica</i>	TSD <sup>amb</sup>
	<i>Melanochelys trijuga</i>	TSD II
	<i>Rhinoclemmys areolata</i>	TSD II <sup>b</sup>
	<i>R. pulcherrima</i>	TSD Ia
FAMILY: Testudinidae		
	<i>Geochelone elephantopus</i>	TSD <sup>amb</sup>
	<i>G. gigantean</i>	TSD <sup>amb</sup>
	<i>Testudo graeca</i>	TSD <sup>amb</sup>
	<i>T. hermanni</i>	TSD <sup>amb</sup>
FAMILY: Chelydridae		
	<i>Chelydra serpentina</i>	TSD II
	<i>Macrolemys temminckii</i>	TSD II
FAMILY: Kinosternidae		

	<i>Kinosternon acutum</i>	TSD <sup>amb</sup>
	<i>K. alamosae</i>	TSD Ia
	<i>K. arizonense</i>	TSD II <sup>b</sup>
	<i>K. baurii</i>	TSD <sup>amb</sup>
	<i>K. creaseri</i>	TSD II
	<i>K. flavescens</i>	TSD <sup>amb</sup>
	<i>K. hirptipes</i>	TSD Ia
	<i>K. leucostomum</i>	TSD <sup>amb</sup>
	<i>K. scorpioides</i>	TSD <sup>amb</sup>
	<i>K. sonoriense</i>	TSD Ia <sup>c</sup>
	<i>K. subrubrum hippocrepis</i>	TSD II <sup>b</sup>
	<i>K. s. subrubrum</i>	TSD Ia <sup>b</sup>
	<i>Sternotherus carinatus</i>	TSD II
	<i>S. minor</i>	TSD II
	<i>S. odoratus</i>	TSD II
FAMILY: Dermatemydidae		
	<i>Dermatemys mawii</i>	TSD <sup>amb</sup>
FAMILY: Cheloniidae		
	<i>Caretta caretta</i>	TSD <sup>amb</sup>
	<i>Chelonia mydas</i>	TSD <sup>amb</sup>
	<i>Eretmochelys imbricate</i>	TSD <sup>amb</sup>
	<i>Lepidochelys kempii</i>	TSD <sup>amb</sup>
	<i>L. olivacea</i>	TSD <sup>amb</sup>
FAMILY: Dermochelyidae		
	<i>Dermochelys coriacea</i>	TSD <sup>amb</sup>
FAMILY: Carettochelyidae		
	<i>Carettochelys insculpta</i>	TSD <sup>amb</sup>
SUBORDER: Pleurodira		
FAMILY: Pelomedusidae		
	<i>Pelomedusa subrufa</i>	TSD II
	<i>Pelusios castaneus</i>	TSD II
	<i>Podocnemis expansa</i>	TSD <sup>amb</sup>

---

\*Data collected from Ewert and Nelson (1991); Janzen and Paukstis (1991a,b); Ewert et al. (2004).

<sup>b</sup>Tendency towards TSD II.

<sup>c</sup>Tendency towards TSD Ia.

<sup>amb</sup>Pattern of TSD ambiguous.