

2011

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Recommended Citation

Warner, Daniel; Mitchell, Timothy; and Janzen, Fredric J. II, "The Effects of Egg-incubation Temperature on Growth and Survival of Hatchling Painted Turtles" (2011). *Iowa State Research Farm Progress Reports*. 188.
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The Effects of Egg-incubation Temperature on Growth and Survival of Hatchling Painted Turtles

Abstract

The environmental conditions that reptile embryos experience during development have profound effects on many offspring traits. For example, incubation temperature affects offspring body size, growth rate, running performance, and even gender—all phenotypic traits that are important for survival and reproduction (i.e., fitness). Although these effects on fitness-related traits are well known, very few studies have evaluated the long-term consequences of incubation temperature on survival and reproduction, particularly in long-lived species.

Keywords

RFR A1009, Ecology Evolution and Organismal Biology

Disciplines

Agricultural Science | Agriculture | Ecology and Evolutionary Biology

The Effects of Egg-incubation Temperature on Growth and Survival of Hatchling Painted Turtles

RFR-A1009

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Introduction

The environmental conditions that reptile embryos experience during development have profound effects on many offspring traits. For example, incubation temperature affects offspring body size, growth rate, running performance, and even gender—all phenotypic traits that are important for survival and reproduction (i.e., fitness). Although these effects on fitness-related traits are well known, very few studies have evaluated the long-term consequences of incubation temperature on survival and reproduction, particularly in long-lived species.

The primary goal of this study was to evaluate the long-term effects of egg-incubation temperature on growth, survival, and reproduction in the painted turtle (*Chrysemys picta*). Additionally, because offspring gender is determined by incubation temperature in *C. picta* (i.e., temperature-dependent sex determination, TSD), our study was also designed to quantify how incubation temperature differentially affects the fitness of male and female offspring. Thus, our results will provide critical insights into the fitness consequences of incubation-induced phenotypic variation and provide a further understanding of the evolution of TSD.

Because *C. picta* reaches sexual maturity at about 5-6 years of age and has a lifespan of

30+ years, our study will continue for multiple years in order to collect reproductive data during a significant subset of the total reproductive lifespan. Our report here provides a preliminary summary of results during the first year of this study.

Materials and Methods

During May and June 2008, forty-three clutches of *C. picta* eggs were collected from our field site at the Thomson Causeway Recreation Area near Thomson, Illinois and Clinton, Iowa. Eggs were transported to Iowa State University (ISU) where they were incubated under three temperature treatments known to produce different offspring sex ratios; a male-producing temperature (26 °C), a temperature that produces balanced sex ratios (28 °C), and a female-producing temperature (30 °C). A subset of eggs in each temperature treatment was given an application of chemicals (estradiol, fadrozole, ethanol) in order to produce both genders at each incubation temperature.

After eggs hatched, turtles were measured, weighed, and released into one of 20 pools (Figure 1a) at the ISU Horticulture Research Station. The hatchling turtles were fed three times per week and monitored closely for any mortality. Dead turtles were identified and preserved in ethanol. On September 26, turtles were collected, measured, and then put into winter hibernation (i.e., placed in water-filled plastic shoeboxes at ~4 °C). Turtles remained in hibernation until March 20, 2009.

To evaluate how incubation temperature affects terrestrial dispersal of hatchling turtles, we constructed 20 circular arenas on flat ground at the ISU Horticulture Research Station (Figure 1b). Arenas were constructed of black weed mat and eight pit traps were

spaced equidistant around the perimeter of each arena. At 0700 hours on April 22, 2009, 11 turtles (i.e., the typical clutch size) were released in the center of each arena. We made sure that each arena contained one turtle from each of our nine incubation treatments and two naturally-incubated hatchlings as controls. The pit traps were checked hourly for the turtles.

Results and Discussion

Overall egg hatching success was 87.1 percent (377 of 433 eggs) and did not differ among incubation temperature treatments ($\chi^2 = 4.8$, $P = 0.089$). Incubation duration differed substantially among treatments ($F = 285.8$, $P < 0.001$); eggs from the warm incubation treatment had a shorter incubation period (mean \pm SE = 54 ± 0.24 days) than those from the intermediate (57 ± 0.26 days) and cool (62 ± 0.26 days) temperature treatments. This result is consistent with past studies showing that warm temperatures accelerate embryonic developmental rate.

Body size of hatchling turtles was significantly affected by incubation temperature (Figure 2a). Hatchling turtles incubated at cool temperatures were longer ($F = 3.0$, $P = 0.050$) and heavier ($F = 3.4$, $P = 0.034$) than those from the intermediate and warm incubation treatments. Post-hatching growth rate of hatchling turtles also differed among incubation treatments (Figure 2b); hatchlings from eggs incubated at warm temperatures grew significantly slower than those from the intermediate and cool temperature treatments. Despite these effects on hatchling size and growth, incubation temperature did not affect survival up to hibernation ($\chi^2 = 6.5$, $P = 0.164$). Overall survival up to hibernation was 69 percent (260 of 377 hatchlings).

Our measurements of post-hibernation hatchling performance during terrestrial dispersal in outdoor arenas were significantly affected by incubation temperature. Turtles

from the warm and intermediate incubation treatments took significantly longer to reach the edge of the arena than those from the cool treatment (Figure 2c; $F = 3.3$, $P = 0.041$). However, despite better performance by cool-incubated turtles, their survival rate was significantly lower than those from the intermediate and warm treatments (Figure 2d; $\chi^2 = 7.9$, $P = 0.019$).

Our preliminary results show that egg-incubation temperature has substantial effects on embryonic development, hatchling size, and growth rate. Although incubation temperature did not impact survival prior to hibernation, the effects on hatchling size and growth may have fitness consequences at an older age. Indeed, we showed that incubation temperature probably has significant impacts on hatchling performance and survival during terrestrial dispersal from a nest site to water. Because the nest environment can affect important phenotypic traits of offspring, nesting female turtles must be under strong selection to choose nest sites with appropriate micro-environments.

This ongoing study will continue to evaluate long-term fitness consequences of the egg-incubation environment. Moreover, once turtles reach sexual maturity, we will be able to identify males and females, which will enable us to quantify the sex-specific effects of incubation temperature on long-term survival and reproduction. This type of analysis will determine if temperature-dependent sex determination ensures that each sex is produced at its own optimal incubation temperature. Thus, our results will provide novel insights into the adaptive value of temperature-dependent sex determination.

Acknowledgements

Thanks to Nick Howell and the staff at the Horticulture Research Station. This project is supported by National Science Foundation grant DEB-0640932.



Figure 1. (a) An array of 20 pools where turtles were raised until hibernation; (b) Three of 20 circular arenas used to measure locomotor and orientation performance after hibernation during terrestrial dispersal of hatchling turtles.

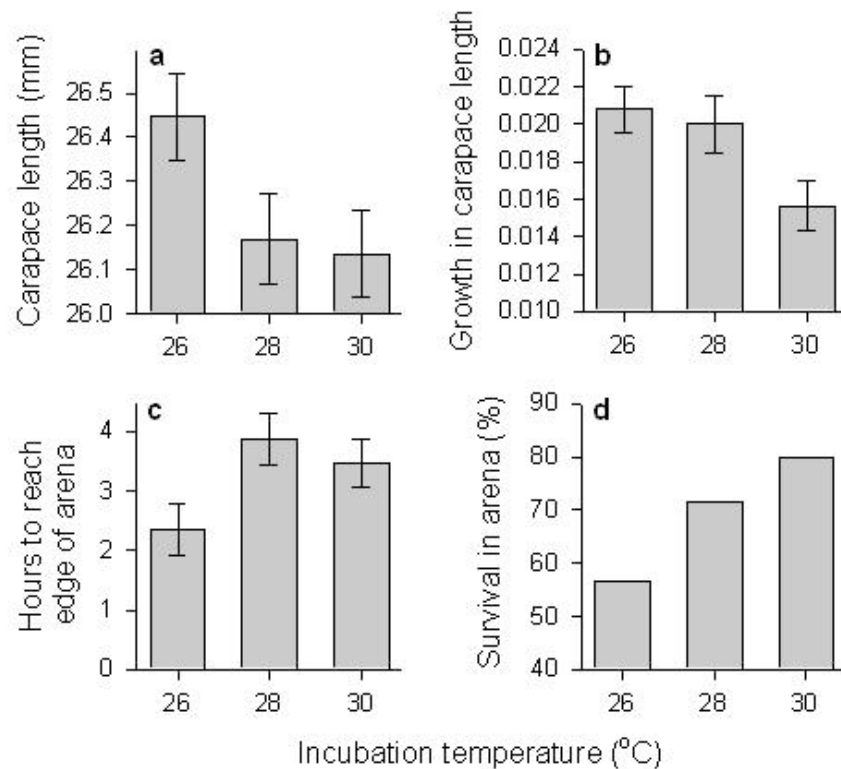


Figure 2. Effects of egg-incubation temperature on hatchling (a) carapace length, (b) growth rate, (c) performance during terrestrial dispersal, and (d) survival during terrestrial dispersal.