

Nest Substrates Influence Nest Temperature and Offspring Sex Ratio in Painted Turtles

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Introduction

Many reptiles have temperature-dependent sex determination (TSD), where the temperature experienced by eggs during incubation determines whether the embryo becomes male or female. Because of this, weather patterns influence the number of males or females being produced in a given year. Over longer time scales, climate changes can produce shifts in population sex ratios, which can be detrimental to population health. Understanding how populations of such reptiles might respond to climate change is important, since most reptiles with TSD are threatened or endangered.

Painted turtles (*Chrysemys picta*) are common in Iowa and have a pattern of TSD where relatively warm temperatures produce females and relatively cool temperatures produce males. In May and June each year, these turtles leave aquatic habitats to dig terrestrial nests and, after laying eggs, permanently abandon the nests. While weather conditions are the primary factor influencing nest temperature, mothers also can influence nest temperatures by choosing where to nest (for example, a shady site will produce a cooler nest than a sunny site). One factor that remains inadequately explored, however, is the influence of soil type on nest temperature. Yet, in many turtle habitats, properties of the soil available for nesting vary substantially. In this experiment, we incubated painted turtle eggs in three ecologically relevant substrate types. We determined if substrate type influenced nest

temperature and characteristics of hatchlings, including sex.

Materials and Methods

In May 2014, we collected 12 clutches of painted turtle eggs from our study population north of Clinton, Iowa along the Mississippi River and transported them to ISU. At the ISU Horticultural Research Station, we constructed six adjacent pits, which were refilled with loamy soil, sand, or gravel. These nesting substrates are commonly selected as nest sites at the field locality where eggs were collected. Artificial nests were created in each substrate, and eggs were assigned to a substrate type in a randomized block design. A temperature logger programmed to record hourly temperatures was inserted into each nest. Eggs remained in these nests for approximately 96 percent of incubation and were retrieved late in development and brought to the lab for hatching. In the lab, we identified the sex of the hatchlings and measured morphological phenotypes, such as body mass and shell length.

Results and Discussion

Substrate type significantly influenced nest temperature. Specifically, loam nests had lower mean daily maxima than gravel or sand nests. Consequently, loam nests also had lower daily means and smaller daily ranges (Table 1). These warmer sand and gravel nests all produced only female offspring, whereas the cooler loam nests produced 70 percent females and 30 percent males. Morphological characteristics of offspring, such as body mass or shell length, did not differ among treatments.

These intriguing results suggest that, indeed, the soil characteristics selected by nesting turtles can influence nest temperature and, in turn, the sex ratio of embryos developing in the nest.

Such information will be critical in parameterizing models that predict population responses to climate change.

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Table 1. Nest temperatures from painted turtle nests incubated in different substrate types.¹

Substrate	Mean (C°)	Minimum (C°)	Maximum (C°)	Range (C°)
Loam	23.8 ^a	19.3	29.2 ^a	9.8 ^a
Gravel	24.3 ^{ab}	18.7	31.2 ^b	12.5 ^b
Sand	24.5 ^b	18.9	31.4 ^b	12.5 ^b

¹Superscript letters indicate significant differences in pairwise statistical comparisons. Means within a column followed by the same letter do not differ ($P \leq 0.05$).