

Chelonian Conservation and Biology, 1999, 3(3):529–531
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A Test of the Comparative Influences of Constant and Fluctuating Incubation Temperatures on Phenotypes of Hatchling Turtles. Linnaeus Fund Research Report

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In the last two decades, numerous researchers have elucidated the effects of incubation temperatures on reptilian embryo survivorship and a variety of hatchling/neonate attributes such as sex, body size, morphology, locomotor performance, energy reserves, and growth rates. These studies have revealed the prevalence of phenotypic plasticity in the reptilian embryo, and a few studies have linked such attributes to hatchling or neonate fitness (e.g., Bennett, 1990; Jayne and Bennett, 1990; Janzen, 1994). It would be valuable to predict distributions of such attributes resulting from natural incubation temperatures. However, egg temperatures in nature fluctuate daily, and the majority of studies investigating relationships between temperature and phenotypic attributes have utilized constant temperatures in the laboratory. This begs three questions: (1) How can we convert fluctuating temperatures into equivalent constant temperatures? (2) Will these “developmental equivalents” produce “phenotypic equivalents?” (3) If not, can results from constant temperature experiments be translated in natural systems?”

The first question has been addressed by Georges (1989) and Georges et al. (1994) by modeling and testing the effects of fluctuating temperatures on sex determination in turtles. Georges et al. (1994) found that the proportion of development, rather than duration of exposure to a certain temperature, predicted offspring sex. They found that the mean of a fluctuating temperature regime did not adequately predict sex or developmental rate, and used the acronym CTE (constant temperature equivalent) to refer to the constant temperature that results in the same overall developmental rate as a given fluctuating regime.

But are hatchlings from fluctuating regimes phenotypically equal to hatchlings from equivalent constant temperature regimes? Would embryos incubated at 30°C constant temperature hatch and perform similarly to those incubated at a fluctuating regime developmentally equivalent to 30°C? If not, how might results of published studies that utilized constant temperatures be

interpreted? How might this direct future research in physiological ecology involving reptilian development?

I carried out an incubation experiment using the softshell turtle, *Apalone spinifera*, in an attempt to answer these questions. I incubated eggs at 10 constant temperatures in the laboratory, and at three fluctuating temperatures in artificial nests in nature. I determined the effects of temperature on developmental rate, hatching success, and hatchling phenotypes: body size, swim speed, endurance, and burying behavior. I used *A. spinifera* because its hard calcareous eggshells render embryos relatively independent of the hydric environment (Packard et al., 1979, 1981), a potentially confounding factor in incubation experiments using turtles with pliable eggshells.

Methods. — I collected *A. spinifera* eggs along the Comite River near Baton Rouge, Louisiana, USA, in July 1995. I used only eggs laid within the last four days, as indicated by patching. Twenty-eight clutches were numbered and split among 13 treatments. Ten treatments were constant temperatures between 25 and 34°C. Three fluctuating temperature treatments were artificial nests on a sandbar along the Comite River, and eggs were buried at depths equaling the mean found for *A. spinifera* at the site (Doody, 1995). Each treatment contained 24 eggs. Constant temperature incubation was carried out in the laboratory using styrofoam incubators (Hovabators™). Field treatments were identified as (1) sunny, (2) semi-shaded, and (3) shaded, and bracketed the thermal environments experienced by *A. spinifera* nests at the site (Doody, 1995). Shading was natural vegetation on sandbars, and sand was transplanted to prevent the potential confounding effect of sand characteristics on temperature. Field nests were caged to protect eggs from predators. Eggs from field treatments were collected just prior to hatching and all eggs were monitored daily for hatching in the laboratory. Hatching turtles were placed in a plastic cup for 14 days to reduce or eliminate acclimatory effects.

On days 14–16 turtles were measured, weighed, and subjected to phenotypic trials. Turtles were encouraged to swim 1 m with the eraser end of a pencil along a track made from a plastic gutter. There were five swim speed trials. Turtles were then flipped onto their backs continually in shallow water until they fatigued. The number of flips was used as an index of endurance. There were four endurance trials. Finally, turtles were placed in shallow water in a small aquarium with a sandy bottom and their burying behavior (measured in propensity and time to bury) observed. There were three burying behavior trials.

I used incubation period to determine which constant temperature treatment was developmentally equivalent to each of the three fluctuating temperature treatments. I hereafter refer to these as overall developmental equivalents (ODE's). Although equations have been constructed to determine CTE's (constant temperature equivalents) of fluctuating temperature regimes (Georges, 1989; Georges et al., 1994), CTE's may or may not be interchangeable with ODE's. Although incubation period was unaffected by fluc-

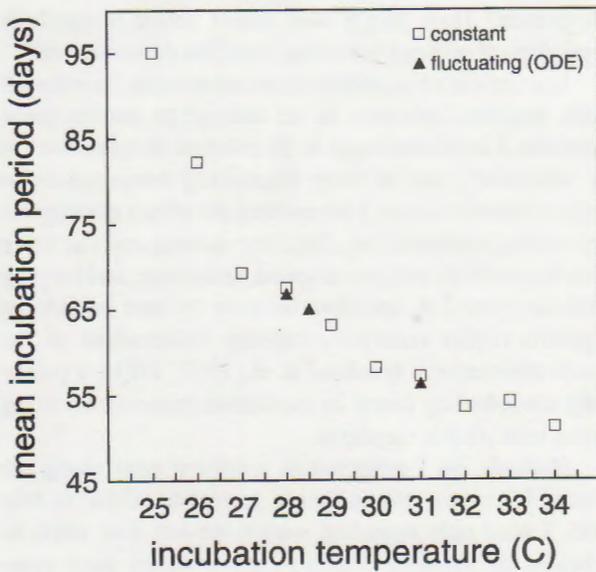


Figure 1. Mean developmental times of softshell embryos from fluctuating temperatures in artificial nests (triangles), plotted along the line representing the relationship between constant incubation temperatures and developmental time in the laboratory (squares). Fluctuating regimes from sunny, semi-shaded, and shaded sites produced incubation periods equivalent to 31, 28.5, and 28°C constant temperature regimes, respectively.

tuations in temperatures in experiments with the turtle *Caretta caretta* (Georges et al., 1994), further research is needed to clarify the relationship between CTE's and ODE's. The null hypothesis, then, was that hatching success and phenotypic performance would be similar between equivalent regime types (ODE's).

Results and Discussion. — Fluctuating regimes from sunny, semi-shaded, and shaded sites produced developmental times equivalent to 31, 28.5, and 28°C constant temperature regimes, respectively (Fig. 1). The shaded site was warmer than anticipated due to late summer senescence

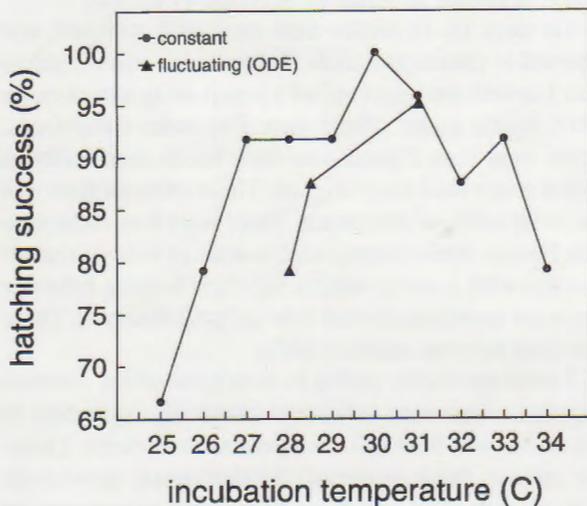


Figure 2. Mean % hatching success of softshell embryos as a function of incubation temperature. Dots denote constant temperature regimes, triangles represent fluctuating regimes converted to constant temperature equivalents using incubation period (see Fig. 1).

of willow trees that shaded the treatment. However, most softshells nest in sunny sites (Webb, 1962). Hatching success did not differ significantly between pairs of developmental equivalents; however, 28°C constant temperature hatchlings experienced higher hatching success than those from the fluctuating shade treatment (Fig. 2). Hatching success differed significantly among treatments within a regime type, yielding a normal distribution (Fig. 2).

Egg mass adjusted hatchling carapace length and plastron length did not differ significantly between pairs of developmental equivalents, but differed significantly among constant temperatures (i.e., smaller hatchlings at higher temperatures), producing a negatively-skewed distribution. Hatchling wet mass was significantly greater in the sunny treatment than its ODE (31°C).

Endurance of hatchlings from the sunny regime was significantly greater than that of hatchlings from its ODE (Fig. 3). Within regime type, constant temperatures produced a leptokurtic (contracted) distribution, peaking at 30°C, while the sunny treatment produced hatchlings with the highest endurance among fluctuating treatments (Fig. 3). No significant differences in burying speed were found between regime types. Within regime types, a bimodal distribution was found for constant temperature hatchlings, with individuals from 26, 27, 31, 32, and 33°C having the highest propensity to bury, while hatchlings from the extremes, and those incubated at 28–30°C were less likely to bury. No significant differences in propensity to bury existed among fluctuating regimes. Hatchlings from the sunny treatment swam significantly slower than hatchlings from its ODE. Hatchlings from 27–29°C constant temperatures and shade and semi-shade fluctuating temperatures swam significantly faster than hatchlings from other treatments within each regime type.

Summary. — The major findings of the study were: (1) Hatchlings from the sunny treatment had higher endurance,

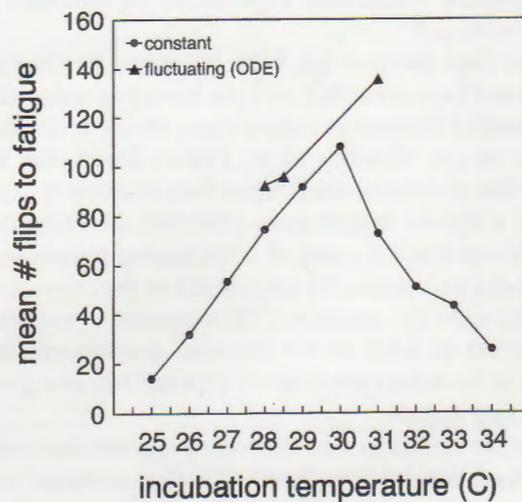


Figure 3. Mean endurance of softshell hatchlings as a function of incubation temperature, measured as the number of flips until turtles fatigued. Dots denote constant temperature regimes, triangles represent fluctuating regimes converted to constant temperature equivalents using incubation period (see Fig. 1).

but were smaller and swam slower than those from the developmentally equivalent constant temperature. (2) Therefore, developmentally equivalent temperature regimes do not necessarily result in offspring with equivalent phenotypes. (3) Because most softshells nest in sunny sites, these results may be interpreted in a natural context. (4) Interpretations stemming from constant temperature incubation studies may need to be re-examined.

The present study was the first designed to test the relative effects of constant and fluctuating temperatures on hatchling phenotypes in reptiles. Findings indicate that interpretations of published incubation studies utilizing constant temperatures may need to be modified. Future incubation studies should incorporate fluctuating temperature regimes, because egg temperatures fluctuate in nature, and because the technology for simulating natural egg temperatures is readily available.

Acknowledgments. — I am grateful to the following for assistance in the field and/or laboratory: C. Shadrix, C. Sheil, L. Turner, and J. Young. The project benefited greatly from conversations with R. Seigel and M. Plummer. Special thanks to G. Childers and the Dept. of Biological Sciences of Southeastern Louisiana University for logistical support. The work reported on herein was supported solely by The Linnaeus Fund of Chelonian Research Foundation.

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Year Funded: 1994

Received: 27 October 1998

Revised and Accepted: 30 July 1999

Chelonian Conservation and Biology, 1999, 3(3):531-533
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Mercury Concentration in the Scutes of Black Sea Turtles, *Chelonia mydas agassizii*, in the Gulf of California.

Linnaeus Fund Research Report

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Anthropogenic mercury contamination of aquatic systems is a serious environmental problem (Laws, 1993), although little is known regarding its accumulation and effects on sea turtles. Research is needed to rapidly and humanely evaluate the effects of mercury and other environmental pollutants on sea turtle health. Mercury and other heavy metals have been found to disrupt normal neurological and physiological function in many species, leading to their stranding, death, and further population decline (Regan et al., 1989; Gottschalk et al., 1991; Colborn et al., 1996). In species in which this biologically nonessential metal has been studied, it impairs growth and reproduction, neurological development and motor coordination, vision, hearing, respiration, blood chemistry, metabolism, and osmoregulation (Eisler, 1987). Additionally, because of its high lipid solubility, mercury has a particular affinity for the central nervous system, where it interrupts the sulfhydryl enzymes of protein synthesis critical to normal neurological function (Osweiler, 1996).

Detection of mercury and other heavy metals in keratinized tissues has been used as an indicator of biotic accumulation in humans (Willhelm et al., 1991), domestic animals (Sakai et al., 1995), and wildlife (Mason et al., 1986). Tissues such as hair, skin, nails, and feathers are rich in sulfhydryl-containing amino acids which avidly bind metals. The carapace of sea turtles is covered by keratinized scutes (Mader, 1996). Collection of keratinized tissue from the scutes of sea turtles has been shown to be a highly sensitive, non-invasive sampling technique for monitoring mercury (Presti et al., in press). It was demonstrated that mercury concentration in the scutes was on average 36 times higher than in the blood. Mercury concentrations in the scutes were also 5.3 times higher than previously reported in the kidney, and 2.5 times higher than in the liver (Landry and