

Does Variation in Soil Water Content Induce Variation in the Size of Hatchling Snapping Turtles (*Chelydra serpentina*)?

MICHAEL S. FINKLER

Most studies that have investigated the influence of hydric conditions during incubation on the size and quality of hatchling reptiles have used either vermiculite or sand as the incubation medium, and the applicability of data derived from eggs using one media type or the other to natural populations has had considerable debate. However, few studies have used soil from actual nesting areas as the incubation medium. In this study, I tested the influence of variation in substrate water content during incubation on the size of hatchling Snapping Turtles from southeastern Michigan using soil collected from actual Snapping Turtle nests. Eggs from six clutches were fully buried in sterilized soil that had been dried and then reconstituted to six different levels of hydration (3, 5, 7, 9, 11, and 13% gravimetric water content). Eggs in the driest soil (3%) had a slight net loss of mass over the course of incubation, whereas eggs in the wetter soils gained mass to varying degrees correlating positively with soil water content. Hatchling mass was significantly lower in the two driest soils than in the wetter soils. Carapace length was significantly shorter for hatchlings from the driest soil (3%) than for those from soils with 7% and 9% water contents, and significantly shorter for hatchlings from the wettest soil (13%) than for those from the 7, 9, and 11% water contents. Soil water contents measured adjacent to a single nest site over three consecutive field seasons ranged from 1.1 to 8.2%, with median water contents $\leq 5\%$ in all three seasons. These findings support the hypothesis that normal variation in soil hydration can influence the size of hatchlings emerging from natural nests. The degree to which hatchling size varies with normal variation in soil water content, however, may have only minor impact on hatchling survivorship in light of other factors that could potentially influence hatchling survival in this species.

THE influence of hydric conditions during incubation on the phenotype of hatchling reptiles has been studied extensively over the past three decades. Studies have been conducted on numerous lizards (M. Packard et al., 1980; Tracy, 1980; Marco et al., 2004), snakes (Gutzke and Packard, 1987; Dmi'el et al., 1993; Ji and Du, 2001), and turtles (Janzen et al., 1995; Hewavithi and Parmenter, 2001; Booth, 2002), but perhaps the most intensively studied species has been the Common Snapping Turtle (*Chelydra serpentina*) of North America. Laboratory studies have demonstrated that eggs incubated under "moist" conditions gain water over the course of incubation, whereas eggs incubated under "dry" conditions lose water. Hatchlings from eggs incubated under moist conditions typically are larger and have smaller post-hatching yolk reserves (Morris et al., 1983), higher metabolic rates (Gettinger et al., 1984; Miller and Packard, 1992), and greater locomotor performance (Miller et al., 1987; Miller, 1993) and desiccation tolerance (Finkler, 1999) than do hatchlings from eggs incubated under dry conditions. Numerous assertions have been made regarding the potential importance of such environmental-ly induced phenotypic variation on the survival

and, ultimately, the fitness of the hatchlings. Field-based studies on *Trachemys scripta* (Janzen et al., 2000a, b) indicate that larger hatchlings have greater survivorship rates during overland migration than do smaller hatchlings, perhaps through greater locomotor performance and an enhanced ability to evade predators. However, the few field-based studies that have been conducted on *C. serpentina* hatchlings have yielded somewhat equivocal evidence as to whether variation in body size or performance influences hatchling survivorship (Janzen, 1993; Congdon et al., 1999; Kolbe and Janzen, 2001).

The majority of studies that have examined the influence of hydric conditions on hatchling phenotype have employed vermiculite as the incubation medium. Some researchers (Ratterman and Ackerman, 1989; Ackerman, 1991; Rimkus et al., 2002), however, have questioned the applicability of incubating eggs in this type of substrate to water relations of eggs in natural nests, noting that variation in soil water content alters both the total water potential of the soil (primarily by altering matric and solute potentials) and the thermal conductivity. Rimkus et al. (2002) used saline solutions of varying osmotic concentration to vary the water potential of sand-

based incubation substrates (through altering solute potential) while maintaining similar substrate thermal conductivities among incubation treatments. They found that embryonic growth appears to be relatively insensitive to variation in total water potential. Thus, observed variation in hatchling phenotype induced in studies that used vermiculite-based incubation media may simply be an artifact of vermiculite's very low thermal conductivity, which would generate artificially high rates of water loss from the eggs compared to more natural substrates with higher thermal conductivities.

Packard et al. (1987), however, found that incubating eggs in sand substrates of varying water contents also induces variation in hatchling size. This suggests that the variation in substrate thermal conductivity brought about by typical levels of variation in substrate water content could influence hatchling phenotype under natural conditions. Moreover, some field-based studies have reported that variation in hydric conditions in the nest lead to variation in hatchling size and body composition (Cagle et al., 1993; Packard et al., 1993, 1999). However, since most studies have measured hydric conditions based on water potential rather than the actual water content of the soil, it is unclear whether the variation in the water content of the substrates (and associated variation in thermal conductivity) is representative of natural soils used for nesting in the field. In the present study, I incubated Snapping Turtle eggs from a population in southeastern Michigan in soil from natural nest sites that had been rehydrated to varying water contents to determine whether variation in water content of natural soil influences hatchling size.

MATERIALS AND METHODS

The eggs used in this study were from a site located on private property near Sylvan, Washtenaw Co., Michigan. Boyer series (0–10% clay and 0.5–3% organic matter at 0–25 cm depth) and Spinks series (2–15% clay and 0.5–3% organic matter at 0–25 cm depth) loamy sands are the predominant soil types at areas where the females nest on the property (USDA, 1977). Eggs were collected from freshly-laid nests at the site on 9 and 10 June 2002. Eggs were placed into damp vermiculite (2 kg water/kg vermiculite) and refrigerated until they were transported to Indiana University Kokomo on 11 June 2002. Soil from 12 separate nests was also collected to serve as the incubation substrate for this experiment.

Upon returning to the laboratory, six different nest substrates were prepared with combined soil

from 12 different nest sites. The soil was autoclaved, dried, then rehydrated to one of six different moisture levels (3, 5, 7, 9, 11, and 13% water content by mass). For comparison with studies that express hydric conditions in terms of water potential, I measured matric water potential at 25 C in three replicates of each soil water content 72 hr after rehydration using a tensiometer (Forestry Suppliers, Inc.). The resultant exponential model generated from these data ($y = -121.74e^{-0.286x}$, $R^2 = 0.969$) was used to calculate the matric water potential in the six different moisture levels (-51.6, -29.1, -16.4, -9.3, -5.2, and -3.0 kPa, respectively). This equation was subsequently used to estimate the matric water potentials of soils monitored in the field.

A total of 120 eggs from six clutches (14–28 eggs from each clutch) were weighed to the nearest 0.001 g, and ten eggs were randomly assigned to one of two nest boxes within each of the six soil moisture levels, with at least one egg from each clutch in each nest box. The eggs were then fully buried at a depth of approximately 3 cm in the soil and incubated at 25 C. Approximately once per week up to day 65 of incubation, eggs in each treatment were weighed to the nearest 0.001 g; eggs demonstrating embryonic mortality (e.g., fungal growth on the outer surface of the egg) were removed, and mass lost from the soil was replaced by adding additional distilled water. Upon hatching, hatchlings ($n = 66$ total) were blotted dry and weighed to the nearest 0.001 g. Hatchlings were housed individually in sample jars containing soil from the nest box for one week post-hatching (to ensure the hatchlings' carapaces had unfolded completely), after which carapace lengths were measured to the nearest 0.1 mm.

To examine variation in soil water content at the nesting areas, samples of soil from 10–15 cm below ground were collected from soil cores taken at areas adjacent to each of the nest sites for the six clutches on 6 September 2002. Variation of soil water content among three incubation seasons (2003–2005) was also examined at a single nest site located on the southeastern face of a hillside with light vegetation approximately 60 m from the nearest permanent water. Soil water content was monitored using paired ECH₂O dielectric soil moisture probes (Decagon Devices, Inc.) connected to a Hobo Micro Station digital datalogger (Onset Computer Corp.). One probe was placed in undisturbed soil within 25 cm of the opening to the nest cavity, whereas the other was inserted at a random location 2 m from the nest. Each probe was inserted fully into the soil, thus

providing average soil water content from 5 to 25 cm below the surface. Soil water content was recorded every five minutes from 16 June–8 August 2003 (terminated early due to equipment malfunction), from 10 June–21 August 2004, and from 13 June–16 August 2005. The BoxCar software (Onset Computer Corp.) used to operate the datalogger expresses soil water content volumetrically (m^3/m^3), so volumetric water contents were measured in tamped soils with 2, 4, 6, 8, 10, 12, and 14% water content by mass to generate a linear model for calculating the gravimetric water content of the soil based on the volumetric water content (gravimetric water content (%) = $27.55 \times \text{volumetric water content (g/g)} + 0.91$, $R^2 = 0.979$).

Egg mass, hatchling mass, and hatchling carapace length were analyzed using the MIXED procedure on SAS 8.02 (Statistical Analysis Systems Institute, Inc., Cary, NC, 2001.). Egg mass during incubation was analyzed using a repeated-measures ANOVA in which incubation treatment (soil water content) was a fixed effect, sampling interval was the repeated effect, and nest box (nested within treatment) and clutch were random effects. Incubation duration, hatchling mass, and carapace lengths were analyzed using ANCOVAs in which soil water content was a fixed effect, nest box (nested within treatment) and clutch were random effects, and initial egg mass was the covariate. The significance of random effects in each model was evaluated by calculating the difference in -2 restricted log-likelihood values between models that included both clutch and nest box as random effects and otherwise identical models that omitted either clutch or nest box nested within treatment. The associated probability for the resultant difference in -2 restricted log-likelihood values (a χ^2 statistic) was then used to assess the importance of the random effect as a covariance parameter in the model (Statistical Analysis Systems Institute, Inc., Cary, NC, 2001.). Since nest box (nested within incubation treatment) was subsequently found to be a very minor, non-significant source of variation for incubation duration, hatchling mass, and carapace lengths (see Results), Type III analyses for the effects of soil water content and initial egg mass on these variables reflect models that do not include nest box. Where appropriate, pairwise comparisons between least-squares means were evaluated based upon t -tests with pairwise error rates of 0.025.

The number of eggs successfully hatching in each treatment was analyzed by combining the successful hatchings in both nest boxes within each treatment. Two different analyses were

performed to assess potential factors influencing hatching success. First, the significance of clutch (a random effect) as a source of variation was evaluated by constructing mixed logistic models using the NLMIXED procedure on SAS 8.02 and comparing the difference in -2 restricted log-likelihood values between a model that included clutch as a random effect and one that did not. Second, differences in hatching success among the soil moisture levels were evaluated using a logistic model generated using the GENMOD procedure. Here, soil water content and clutch were included in the model as fixed effects, though only the results pertaining to the influence of soil water content are reported herein.

RESULTS

The water contents of soil samples obtained from six nest sites near the end of the incubation were 2.2, 2.6, 3.0, 3.4, 5.7, and 6.2% water by mass (mean 3.9%). A somewhat broader range of water contents was recorded at a single nest site in the field during the three summers monitored (1.1–8.2% over the three field seasons, Fig. 1). There was considerable variation in the water contents among years, with the overall highest water contents measured in 2004 (median 5.0%, min 3.3%, max 8.2%), the lowest measured in 2005 (median 2.2%, min 1.1%, max 5.8%), and intermediate water contents measured in 2003 (median 2.7%, min 1.9%, max 6.2%). Estimated water potential in the field ranged from -20.4 to -70.7 kPa (median -57.1 kPa) in 2003, from -11.7 to -48.1 kPa (median -28.9 kPa) in 2004, and from -23.0 to -90.2 kPa (median -65.1 kPa) in 2005.

Clutch was a significant source of variation in the pattern of egg mass change over the course of incubation, and for both hatchling mass and carapace length (Table 1). However, clutch was not a significant source of variation in incubation duration. Nest box (nested within treatment) was only a significant source of variation in the pattern of egg mass change during the course of incubation.

Changes in egg mass during incubation differed among the different treatments (Fig. 2, Table 2). Eggs in the driest treatment (3%) generally demonstrated a slight net loss in mass during incubation, whereas eggs in the remaining treatments gained mass to varying degrees during incubation that correlated linearly with water content of the soil.

Hatching success differed significantly among the soil water contents. The number of successful hatches in each group (out of 20 eggs beginning incubation in each group) were as follow: 3%: n

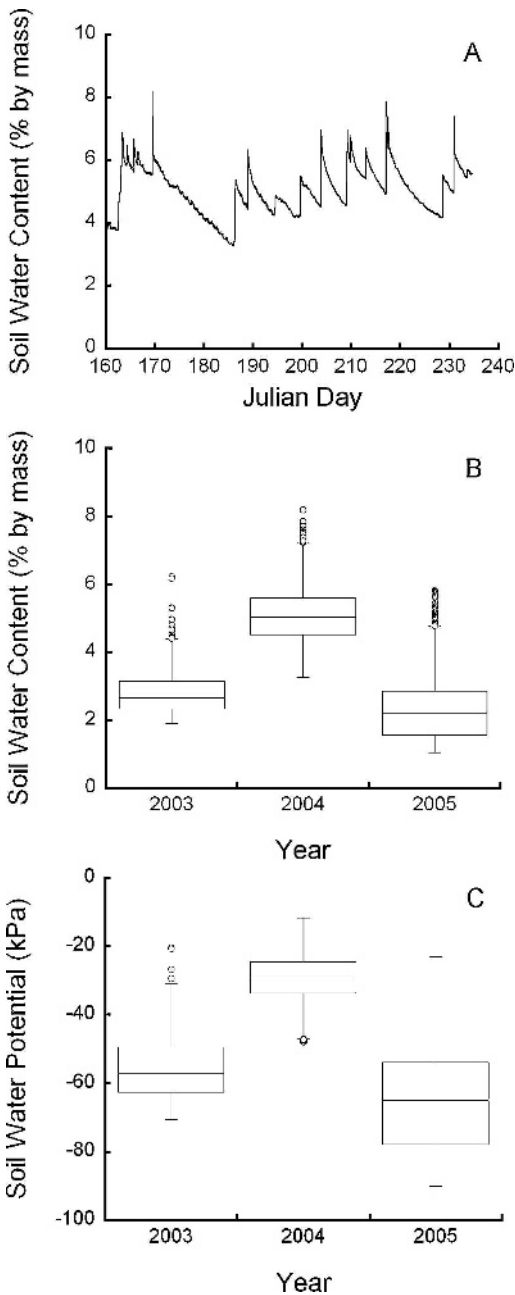


Fig. 1. Variation in water content and estimated water potential at a single nest site over a three-year period. (A) Example of soil water content (% water by mass) recording from single nest site during the 2004 field season. Data depicted are averaged between two dielectric soil moisture probes associated with the nest site. (B) Box and whisker plots describing variation in soil water content averaged between two dielectric soil moisture probes at same nest site among three different field seasons (2003–2005). Each box encloses 50% of the data with the median value of the variable displayed as a line. Top

= 9; 5%: $n = 10$; 7%: $n = 14$; 9%: $n = 16$; 11%: $n = 9$; 13%: $n = 8$. Survivorship was significantly lower in the 13% treatment than in either the 7% treatment or the 9% treatment ($\chi^2 > 5.02$, $df = 1$, $P < 0.025$). Hatching success was not significantly influenced by clutch of origin ($\chi^2 = 0.9$, $df = 5$, $P = 0.97$).

Soil water content significantly influenced the duration of incubation (Table 2, Fig. 3). Eggs incubated in 13% soil took significantly longer to hatch than did those incubated in drier soils.

Hatchlings from eggs incubated in both 3% and 5% soils had significantly lower masses than did hatchlings from the 7% and 11% soils, and those from the 3% soil were also significantly less massive than those from the 9% soil ($P < 0.025$; Fig. 3, Table 2). Hatchlings from the 3% treatment had significantly shorter carapace lengths than did hatchlings from the 7% and 9% treatments ($P < 0.025$; Fig. 3, Table 2). Hatchlings from the 13% treatment had significantly shorter carapace lengths than did those from the 7, 9, and 11% treatments.

DISCUSSION

There has been considerable and lively discussion regarding factors affecting water exchange between the substrate of reptile nests and the eggs within, and the effects such exchanges have on embryonic development and the phenotype of the hatchling. Water exchange between the nest substrate and the eggs within the nest can be influenced by numerous factors, including water potential (G. Packard et al., 1980, 1987; Rimkus et al., 2002), ambient temperature (Packard et al., 1987), metabolic heat production by the developing embryo (G. Packard et al., 1980; Gettinger et al., 1984), the degree to which the eggs are in contact with the incubation substrate

and bottom of the box mark limits of $\pm 25\%$ of the variable population; “whiskers” extending from top and bottom of each box mark minimum and maximum values that fall within $1.5 \times$ the interquartile distance (difference between upper and lower quartiles). Any value outside of this range (an outlier) is displayed as an individual point. (C) Box and whisker plots depicting variation in estimated soil water potential for nest site among three different field seasons (2003–2005). Soil water potential estimated from exponential curve (soil water potential = $-121.74e^{-0.286x}$, $R^2 = 0.969$) generated from measurements of water potential in soils with different gravimetric water contents at 25 C.

TABLE 1. EVALUATIONS OF SIGNIFICANCE OF CLUTCH AND NEST BOX (BOTH TREATED AS RANDOM EFFECTS) IN MIXED MODELS EXAMINING VARIATION IN *C. serpentina* EGG MASS DURING INCUBATION, INCUBATION DURATION, AND HATCHLING SIZE.

Variable	Factor	Variance estimate	χ^2	df	P
Egg mass	Clutch	0.882	58.0	5	<0.0001
	Nest box (Treatment)	0.131	37.5	11	<0.0001
Incubation duration	Clutch	0.971	6.6	5	0.25
	Nest box (Treatment)	<0.001	<0.1	11	>0.99
Hatchling mass	Clutch	0.560	24.1	5	<0.0001
	Nest box (Treatment)	<0.001	<0.1	11	>0.99
Carapace length	Clutch	1.310	28.8	5	<0.0001
	Nest box (Treatment)	0.122	0.8	11	>0.99

(cf. Packard et al., 1987; Rimkus et al., 2002), and the thermal conductivity of the nest substrate (Ackerman et al., 1985; Kam and Ackerman, 1990; Ackerman, 1991). Both the water potential and the thermal conductivity of the nest substrate are influenced by the water content of the substrate, and therefore fluctuations in soil water content (e.g., from variation in precipitation, soil drainage, evaporation, etc.) would alter both properties simultaneously in natural nests (Rimkus et al., 2002).

Previous studies have demonstrated that eggs incubated in substrates with low thermal conductivities (e.g., vermiculite) and/or with a relatively low proportion of the egg surface in direct contact with the substrate (e.g., half-buried eggs) tend to show more curvilinear changes in egg mass, with a notable decrease in egg mass

towards the latter part of incubation (G. Packard et al., 1980; Morris et al., 1983; Packard et al., 1987). This is presumably due to elevated heat generation by the embryo and the associated change in the vapor pressure of the egg relative to that of the substrate (G. Packard et al., 1980; Ackerman, 1994). In contrast, eggs fully buried in soils with higher thermal conductivities (e.g., sand) tend to show more linear changes in mass over the course of incubation (Kam and Ackerman, 1990; Rimkus et al., 2002). Moreover, eggs incubated in substrates with higher thermal conductivities tend to gain more water than eggs incubated in substrates with lower thermal conductivities but similar matric water potentials (Kam and Ackerman, 1990).

In this study, egg mass changed over the course of incubation in a manner consistent with studies that used sand as an incubation medium and where the eggs were fully-buried in the incubation medium (Kam and Ackerman, 1990; Rimkus et al., 2002). Eggs in all but the driest treatment (3%) demonstrated a net increase in mass over the course of incubation. Moreover, eggs in all but the two driest treatments (3% and 5%) demonstrated increases in the rate of water uptake during the latter part of incubation. Rimkus et al. (2002) noted similar increases in humid treatments, and ascribed this to increased surface area of the eggs and a thinning and exfoliating of the egg shell as the volume of the egg increases.

In this study, soil with a 3% water content had an associated water potential of approximately -50 kPa, and eggs incubated in this soil demonstrated a net mass loss. This is in contrast to other studies have found that eggs incubated in vermiculite substrates with a water potential of approximately -50 kPa tended to increase in mass over the course of incubation (e.g., Kam and Ackerman, 1990). One possible explanation for this may be the impact of different proportions of water present in the soil on the thermal

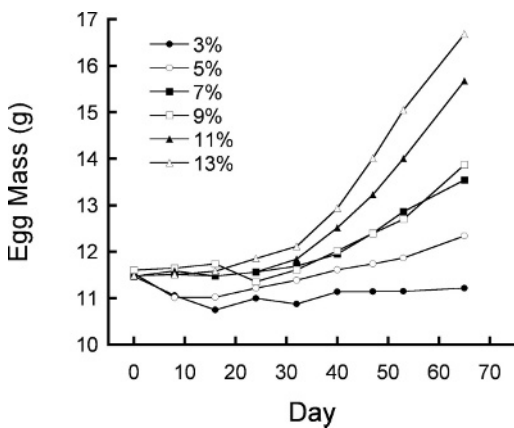


Fig. 2. Changes in mean mass during incubation for eggs incubated in soil of six different water contents at 25 C. Only eggs that survived to day 65 of incubation are included. Number of eggs in each soil moisture content are as follow: 3%: n = 12; 5%: n = 17; 7%: n = 17; 9%: n = 17; 11%: n = 19; 13%: n = 14.

TABLE 2. TYPE III TESTS EXAMINING THE INFLUENCE OF NEST SOIL WATER CONTENT ON VARIATION IN SNAPPING TURTLE EGG MASS DURING INCUBATION, INCUBATION DURATION, AND HATCHLING SIZE.

Variable	Factor	F	df	P
Egg mass	Soil water content	6.23	5,6	0.023
	Sampling interval	327.78	8,798	<0.001
	Soil water content × Sampling interval	48.63	40,798	<0.001
Incubation duration	Soil water content	7.44	5,53	<0.001
	Initial egg mass	4.29	1,53	0.043
Hatchling mass	Soil water content	3.73	5,53	0.006
	Initial egg mass	1.87	1,53	0.18
Carapace length	Soil water content	3.08	5,53	0.017
	Initial egg mass	4.43	1,53	0.040

conductivity of the incubation medium. Kam and Ackerman (1990) reported that a vermiculite substrate formed by combining equal masses water and dry vermiculite (with a bulk density of 0.10 g/cm^3 and a water potential of -55 kPa) had a thermal conductivity of $0.55 \text{ W} \cdot \text{m}^{-1} \cdot \text{K}^{-1}$, which is not much lower than the thermal conductivity of liquid water (approximately $0.6 \text{ W} \cdot \text{m}^{-1} \cdot \text{K}^{-1}$, Andersland and Ladanyi, 1994). Natural soils can vary considerably in their thermal conductivity (0.15 to $1.5 \text{ W} \cdot \text{m}^{-1} \cdot \text{K}^{-1}$, Andersland and Ladanyi, 1994) depending upon a variety of factors, including particle size, organic content, salt concentration, bulk density, and moisture content (Abu-Hamdeh and Reeder, 2000). Although the thermal conductivities of the soils used in this study were not measured, Abu-Hamdeh and Reeder (2000) reported that sandy loam soils with water contents of $\leq 9.4\%$ and bulk densities of $\leq 1.32 \text{ (g/cm}^3)$ had thermal conductivities lower than $0.55 \text{ W} \cdot \text{m}^{-1} \cdot \text{K}^{-1}$. Thus, the thermal conductivities of some of the soils used in this study may have been lower than those of moist vermiculite substrates used in other studies.

The live mass of the hatchlings varied with incubation substrate water content below 7%, but did not differ with substrate water contents between 7% and 11%. These findings are consistent with those of Rimkus et al. (2002), who found that hatchling size was largely independent of net water exchange during incubation in excess of +10% of initial egg mass, but smaller increases in egg mass or decreases in egg mass led to decreased hatchling size. Moreover, hatchlings emerging from eggs in the driest treatment (in which eggs generally demonstrated a net loss of mass) had significantly lower carapace lengths at one week post-hatching than did hatchlings from soil water contents of 7% and 9%, which demonstrated gains in egg mass in excess of +10% initial mass. This suggests that variation in hatchling size

among the drier treatment groups was not simply due to variation in the water content of the hatchlings, but also the result of differential tissue growth. Similarly, Packard et al. (1999) found that wetter nest environments (based on soil water potential) yielded hatchlings that were not only more massive, but had greater dry carcass masses, indicating greater embryonic tissue growth. Rimkus et al. (2002) also noted that hatchling carcass dry mass was reduced when eggs demonstrated less than a +10% gain in mass from initial egg mass.

Curiously, hatchlings from the wettest soil (13%) had significantly smaller carapace lengths in comparison to hatchlings reared in soils from 7–11% water content. This could be due to the reduced gas conductivity of the soil as it approaches saturation, impeding oxygen delivery to the developing embryos (Ackerman 1977; Kam, 1993; Booth, 1998), particularly in the latter portions of incubation when critical oxygen tensions for the embryos are elevated (Kam, 1993; Kam and Lillywhite, 1994). The comparatively low hatching success and the longer duration of incubation prior to hatching in this group support this hypothesis. Moreover, although Packard and Packard (1989) found that calcium mobilization into the embryo from the shell was higher in moister vermiculite substrates, Bilinski et al. (2001) reported that rates of calcium mobilization were higher in eggs incubated in sand with a 2% water content than in those incubated in sand with a 12% water content. This suggests that the very high water content in the 13% treatment may have contributed to reduced incorporation of calcium into the bone and other tissues of the embryos, perhaps through a leaching of calcium out of the eggshell into the surrounding soil.

In general, the findings of this study support the assertions of Rimkus et al. (2002) that in substrates with high thermal conductivity, such as the natural soils in which Snapping Turtles

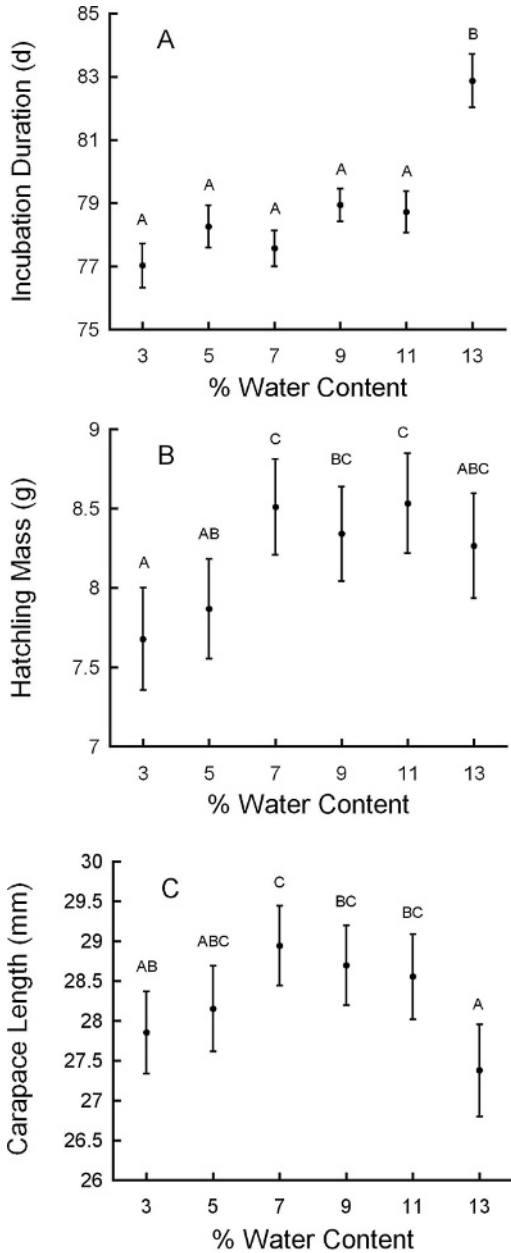


Fig. 3. (A) Duration of incubation, (B) live mass at hatching, and (C) carapace lengths at one week post-hatching for hatchlings from eggs incubated in soils of different water contents (by mass). Data are presented as least-squares means \pm SEM. Like letters indicate no significant difference between least-squares means ($P > 0.025$). Sample sizes in each treatment are as follow: 3%: $n = 9$; 5%: $n = 10$; 7%: $n = 14$; 9%: $n = 16$; 11%: $n = 9$; 13%: $n = 8$.

typically nest, changes in egg mass tend to be more positive and more linear over the course of incubation than in substrates with low thermal

conductivities such as vermiculite, and hatchling size is largely independent of water gain by the eggs during incubation once the amount of water gained is in excess of 10% initial egg mass. However, the findings of this study do not support the suggestion of Rimkus et al. (2002) that hydration states in the nest would rarely influence hatchling size. The water content of soil used in this study would need to be in excess of 5% to induce an average change in egg mass of +10% or more during the course of incubation. Both the soil cores from multiple nest sites near the end of incubation and readings from dielectric probes at a single nest site over three seasons indicated that soil water content was typically below 5%. Although typical levels of variation in matric water potential in natural nests may not have appreciable influence on embryonic development and hatchling phenotype, the data in this study suggest that for this population natural variation in soil water content appears to be sufficient to induce variation in the size of the hatchlings through the associated change in thermal conductivity. However, since these readings were taken from soil adjacent to nest sites and not actually inside the nests, they do not reflect additional intrinsic factors that could modify the water content of the soil during incubation, such as fluid deposited in the nest by the female upon oviposition (Kinney et al., 1998) or exchanges of water among eggs within the clutch and between the entire clutch and surrounding substrate (Gutzke, 1984; Hotaling et al., 1985). It is particularly noteworthy that hatchling survivorship was highest at soil water contents of 7–9% despite the fact that field measurements of soil water content were usually well below 7%. Further examination of changes within the water content of the substrata of complete nests is needed to ascertain the impact of such factors on hatchling phenotype.

The present study indicates that variation in the water content in natural soils likely induces variation in hatchling size. However, although some studies have suggested that larger hatchling turtles may have higher survivorship during periods such as overland movement to water following nest emergence (e.g., Janzen et al., 2000a, b), the degree of variation in hatchling size observed in this study may have relatively little impact on the life history and ecology of Snapping Turtles in this region of the species range. In a series of experiments conducted at a site approximately 24 km northeast from this study site, Congdon et al. (1999) found little evidence of directional selection favoring larger hatchlings over smaller hatchlings, either in

three short-term (one season) experimental releases/recaptures or in a fifteen-year examination of hatchling survival (to at least one year of age) despite considerable variation in hatchling size (6–12 g live mass, 26–33 mm carapace length). In light of this study, it appears that the degree of variation in hatchling size induced by natural degrees of variation in soil water content would have little impact on survivorship of hatchlings (at least those from southeastern Michigan), particularly in comparison to other factors such as the slope of the terrain and the density of vegetation between the nest site and the water (Kolbe and Janzen, 2001).

ACKNOWLEDGMENTS

Eggs were collected with the permission of the Michigan Department of Natural Resources. This study was conducted with the approval of the Indiana University at Kokomo IACUC (Protocol 99-6). I thank D. Schultz and R. Schultz for allowing me to conduct this research on their property and for their assistance in collecting eggs and setting up the dataloggers, and I thank E. Dzialowski and T. Blank for suggestions that helped improve this manuscript.

LITERATURE CITED

- ABU-HAMDEH, N. H., AND R. C. REEDER. 2000. Soil thermal conductivity: effects of density, moistures, salt concentration, and organic matter. *Soil Sci. Soc. Am. J.* 64:1285–1290.
- ACKERMAN, R. A. 1977. The respiratory gas exchange of sea turtle nests (*Chelonia*, *Caretta*). *Resp. Physiol.* 31:19–38.
- . 1991. Physical factors affecting the water exchange of buried reptile eggs, p. 193–212. *In: Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*. D. C. Deeming and M. J. W. Ferguson (eds.). Cambridge Univ. Press, New York.
- . 1994. Temperature, time and reptile water exchange. *Isr. J. Zool.* 40:293–306.
- , R. SEAGRAVE, R. DMI'EL, AND A. AR. 1985. Water and heat exchange between parchment-shelled reptile eggs and their surroundings. *Copeia* 1985:703–711.
- ANDERSLAND, O. B., AND B. LADANYI. 1994. *An Introduction to Frozen Ground Engineering*. Chapman and Hall, New York.
- BILINSKI, J. J., R. D. REINA, J. R. SPOTILA, AND F. V. PALADINO. 2001. The effects of nest environment on calcium mobilization by leatherback turtle embryos (*Dermochelys coriacea*) during development. *Comp. Biochem. Physiol. A* 130:151–162.
- BOOTH, D. T. 1998. Nest temperature and respiratory gases during natural incubation in the broad-shelled river turtle, *Chelodina expansa* (Testudinata: Chelidae). *Austr. J. Zool.* 46:183–191.
- . 2002. Incubation of rigid-shelled eggs: do hydric conditions matter? *J. Comp. Physiol. B* 172: 627–633.
- CAGLE, K. D., G. C. PACKARD, K. MILLER, AND M. J. PACKARD. 1993. Effects of the microclimate in natural nests on development of embryonic painted turtles, *Chrysemys picta*. *Funct. Ecol.* 7:653–660.
- CONGDON, J. D., R. D. NAGLE, A. E. DUNHAM, C. W. BECK, O. M. KINNEY, AND S. R. YEOMANS. 1999. The relationship of body size to survivorship of hatchling snapping turtles (*Chelydra serpentina*): an evaluation of the “bigger is better” hypothesis. *Oecologia* 121:224–235.
- DMI'EL, R., G. PERRY, A. BELINSKI, AND R. A. ACKERMAN. 1993. The effects of hydric and thermal properties of incubation substrate on embryonic development in the water snake, *Natrix tessellata*. *Herpetol. J.* 3:60–64.
- FINKLER, M. S. 1999. Influence of water availability during incubation on hatchling size, body composition, desiccation tolerance and terrestrial locomotor performance in the snapping turtle, *Chelydra serpentina*. *Physiol. Biochem. Zool.* 72:714–722.
- GETTINGER, R. D., G. L. PAUKSTIS, AND W. H. N. GUTZKE. 1984. Influence of hydric environment on oxygen consumption by embryonic turtles *Chelydra serpentina* and *Trionyx spiniferous*. *Physiol. Zool.* 57:468–473.
- GUTZKE, W. H. N. 1984. Modification of the hydric environment by eggs of snapping turtles (*Chelydra serpentina*). *Can. J. Zool.* 62:2401–2403.
- , AND G. C. PACKARD. 1987. Influence of the hydric and thermal environments on eggs and hatchlings of bull snakes *Pituophis melanoleucus*. *Physiol. Zool.* 60:9–17.
- HEWAVISENTHI, S., AND C. J. PARMENTER. 2001. Influence of incubation environment on the development of the flatback turtle (*Natator depressus*). *Copeia* 2001:668–682.
- HOTALING, E. C., D. C. WILHOFT, AND S. B. MCDOWELL. 1985. Egg position and weight of hatchling snapping turtles, *Chelydra serpentina*, in natural nests. *J. Herpetol.* 19:534–536.
- JANZEN, F. J. 1993. An experimental analysis of natural selection on body size of hatchling turtles. *Ecology* 74:332–341.
- , J. C. AST, AND G. L. PAUKSTIS. 1995. Influence of the hydric environment and clutch on eggs and embryos of two species of sympatric map turtles. *Funct. Ecol.* 9:913–922.
- , J. K. TUCKER, AND G. L. PAUKSTIS. 2000a. Experimental analysis of an early life-history stage: selection on size of hatchling turtles. *Ecology* 81:2290–2304.
- , ———, AND ———. 2000b. Experimental analysis of an early life-history stage: avian predation selects for larger body size of hatchling turtles. *J. Evol. Biol.* 13:947–954.
- Ji, X., AND W. G. DU. 2001. Effects of thermal and hydric environments on incubating eggs and hatchling traits in the cobra, *Naja naja*. *J. Herpetol.* 35:186–194.

- KAM, Y.-C. 1993. Physiological effects of hypoxia on metabolism and growth of turtle embryos. *Resp. Physiol.* 92:127–138.
- , AND R. A. ACKERMAN. 1990. The effect of incubation media on the water exchange of snapping turtle (*Chelydra serpentina*) eggs and hatchlings. *J. Comp. Physiol. B* 160:317–324.
- , AND H. B. LILLYWHITE. 1994. Effects of temperature and water on critical oxygen tension of turtle embryos. *J. Exp. Zool.* 268:1–8.
- KINNEY, O. M., R. D. NAGLE, AND J. D. CONGDON. 1998. Water transport by nesting painted turtles (*Chrysemys picta marginata*) in Michigan. *Chel. Conserv. Biol.* 3:71–76.
- KOLBE, J. J., AND F. J. JANZEN. 2001. The influence of propagule size and maternal nest-site selection on survival and behaviour of neonate turtles. *Funct. Ecol.* 15:772–781.
- MARCO, A., C. DÍAZ-PANIAGUA, AND J. HIDALGO-VILA. 2004. Influence of egg aggregation and soil moisture on incubation of flexible-shelled lacertid lizard eggs. *Can. J. Zool.* 82:60–65.
- MILLER, K. 1993. The improved performance of snapping turtles (*Chelydra serpentina*) hatched from eggs incubated on a wet substrate persists through the neonatal period. *J. Herpetol.* 27:233–236.
- , AND G. C. PACKARD. 1992. The influence of substrate water potential during incubation on the metabolism of embryonic snapping turtles (*Chelydra serpentina*). *Physiol. Zool.* 65:172–187.
- , ———, AND M. J. PACKARD. 1987. Hydric conditions during incubation influence locomotor performance of hatchling snapping turtles. *J. Exp. Biol.* 127:401–412.
- MORRIS, K. A., G. C. PACKARD, T. J. BOARDMAN, G. L. PAUKSTIS, AND M. J. PACKARD. 1983. Effect of the hydric environment on growth of embryonic snapping turtles (*Chelydra serpentina*). *Herpetologica* 39:272–285.
- PACKARD, G. C., K. MILLER, AND T. J. BOARDMAN. 1987. Influence of moisture, temperature and substrate on snapping turtle eggs and embryos. *Ecology* 68:983–993.
- , ———, AND M. J. PACKARD. 1993. Environmentally induced variation in body size of turtles hatching in natural nests. *Oecologia* 93:445–448.
- , ———, ———, AND G. F. BIRCHARD. 1999. Environmentally induced variation and condition in hatchling snapping turtles (*Chelydra serpentina*). *Can. J. Zool.* 77:278–289.
- , T. L. TAIGEN, M. J. PACKARD, AND T. J. BOARDMAN. 1980. Water relations of pliable-shelled eggs of common snapping turtles (*Chelydra serpentina*). *Ibid.* 58:1404–1411.
- PACKARD, M. J., AND G. C. PACKARD. 1989. Environmental modulation of calcium and phosphorus metabolism in embryonic snapping turtles *Chelydra serpentina*. *J. Comp. Physiol. B* 159:501–508.
- , ———, AND T. J. BOARDMAN. 1980. Water balance of the eggs of a desert lizard (*Callisaurus draconoides*). *Can. J. Zool.* 58:2051–2058.
- RATTERMAN, R. J., AND R. A. ACKERMAN. 1989. The water exchange and hydric microclimate of painted turtle (*Chrysemys picta*) eggs incubating in field nests. *Physiol. Zool.* 47:1059–1079.
- RIMKUS, T. A., N. HRUSKA, AND R. A. ACKERMAN. 2002. Separating the effects of vapor pressure and heat exchange on water exchange by snapping turtle (*Chelydra serpentina*) eggs. *Copeia* 2002:706–715.
- TRACY, C. R. 1980. Water relations of parchment-shelled lizard (*Sceloporus undulatus*) eggs. *Ibid.* 1980:478–482.
- USDA. 1977. Soil Survey of Washtenaw County, Michigan. United States Department of Agriculture, Soil Conservation Service, Washington, D.C.
- DEPARTMENT OF NATURAL, INFORMATION, AND MATHEMATICAL SCIENCES, INDIANA UNIVERSITY AT KOKOMO, KOKOMO, INDIANA 46904-9003. E-mail: mfinkler@iuk.edu. Submitted: 4 Dec. 2005. Accepted: 31 May 2006. Section editor: R. Mason.