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Microgeographic Variation in Response of Red-eared Slider (*Trachemys scripta elegans*) Embryos to Similar Incubation Environments

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ABSTRACT.—We examined site-specific variation in the response of red-eared slider (*Trachemys scripta elegans*) embryos exposed to similar incubation environments, and collected at five nearby sites in west-central Illinois. Overall, site was not a significant source of variance in change in egg mass during incubation, in hatchling wet mass, and in hatchling carapace length. However, site was a significant source of variance in incubation period. Nonetheless, significant site-specific differences in each trait were detected in pairwise comparisons. The actual difference between extremes was small. Eggs from the site with the longest incubation period also gained the most water during incubation. Our study has important implications for future studies of geographic variation in the physiological response of embryos to incubation environments. Comparisons between eggs and embryos from geographically distant sites would benefit by inclusion of as many clutches as possible. Larger numbers of clutches reduce the possibility that any differences between geographically distant regions are due to maternal differences rather than region-specific differences. Studies comparing embryonic responses from geographically distant regions would be strengthened by including turtles from as many local collecting sites for each region as possible. Sampling a single site per region may be inadequate because any geographic variation in embryonic response could just as well be due to undetected local site-specific differences.

Turtles exhibit geographic variation in many morphological traits (see Ernst et al., 1994, for review). Moreover, life history traits such as egg size and clutch size also vary geographically among turtle species (reviewed by Fitch, 1985; Iverson et al., 1993; Ernst et al., 1994). In contrast, egg incubation and the response of embryos to incubation conditions has been little studied within a geographic context. Geographic variation in incubation periods is known to occur in the common snapping turtle, *Chelydra serpentina* (Ewert, 1985; Iverson et al., 1997). However, nothing is known about possible geographic variation in other responses of turtle embryos to incubation environments.

Turtle embryos contained in flexible-shelled eggs display predictable phenotypic responses to hydric and thermal environments during incubation (reviewed by Packard, 1991). These responses have important effects on hatchling size, on the amount of residual yolk, and on incubation period. Consequently, eggs and embryos might be expected to be adapted to maximize their performance to the hydric and thermal regimes characteristic of the geographic location of the nest site. Therefore, geographic variation in these traits would be expected to occur. The study of geographic variation in any trait is simplified when the extent to which a trait varies within and among populations from the same area is known.

In the current study, we examine variation in the response of red-eared slider (*Trachemys scripta elegans*) embryos exposed to similar incubation environments, and collected at five nearby sites in west-central Illinois. This turtle is a good subject for such a study because it is a widely distributed, polytypic species (Ernst and Barbour, 1989; Ernst et al., 1994) that lays flexible-shelled eggs (Congdon and Gibbons, 1985) sensitive to incubation environments (Congdon and Gibbons, 1990; Tucker et al., 1997, 1998a). Moreover, its biology has been studied extensively in the southeastern United States (reviewed in Gibbons, 1990) and elsewhere (Cagle, 1950; Moll and Legler, 1971; Thornhill, 1982; Moll and Moll, 1990; Tucker and Moll, 1997; Tucker, 1997).

Trachemys scripta is also a good choice to study variation among nearby populations (=microgeographic variation) because other studies have exposed considerable microgeographic variation in other traits. For instance, microgeographic variation has been found in reproduction and growth (Gibbons, 1967; Gibbons and Tinkle, 1969; Tucker et al., 1998c), in age
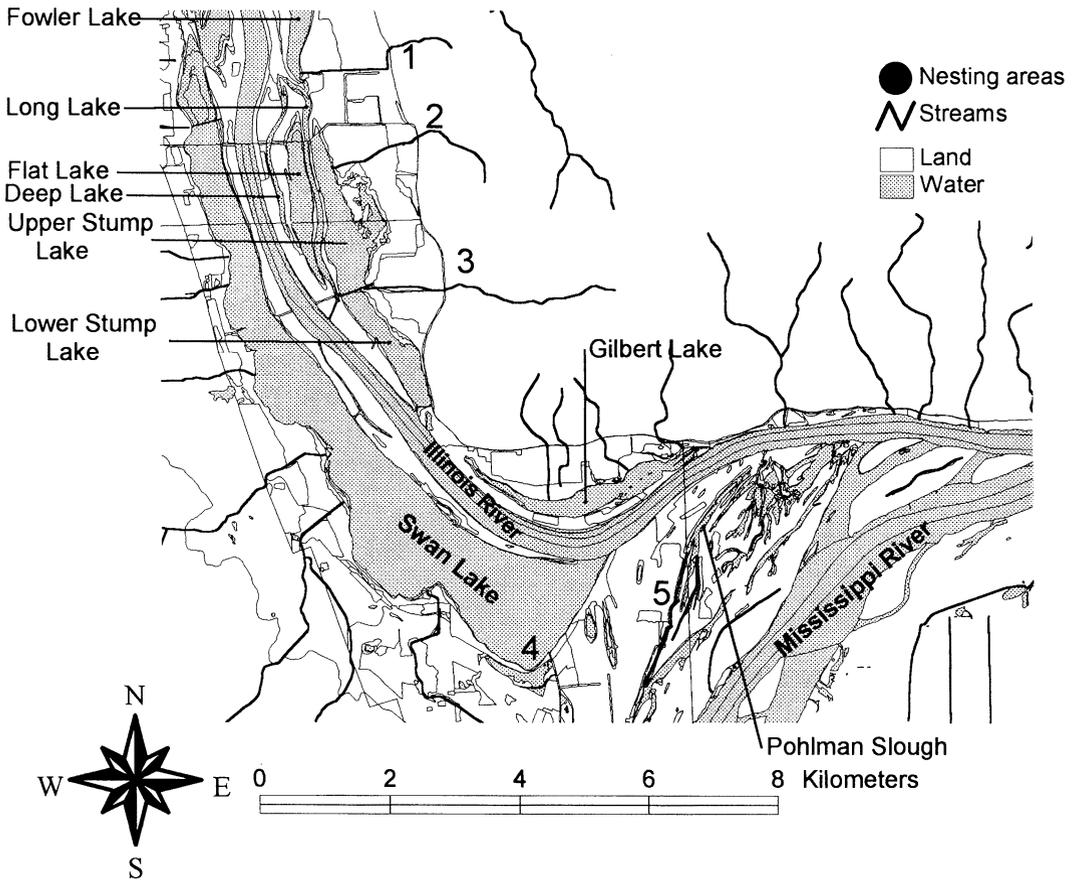
FIG. 1. Location of five nesting areas in west-central Illinois where nesting female red-eared sliders (*Trachemys scripta elegans*) were collected. Location 1 is near Fowler Lake, location 2 is near Upper Stump Lake, location 3 is near Lower Stump Lake, location 4 is near Swan Lake, and location 5 is near Pohlman Slough.

and size at maturity (Gibbons et al., 1981), in carapace morphology (Tucker et al., 1998b), in Mendelian inherited proteins (reviewed by Smith and Scribner, 1990), and in the development of melanism (Lovich et al., 1990; Tucker et al., 1995a). Our experiment is the first to examine embryonic response for microgeographic variation. Such studies of site-specific variation from nearby sites are needed for comparison to future studies of geographic variation among distant sites.

METHODS AND MATERIALS

Collecting Sites.—Females were collected at nesting areas near Pohlman Slough (Sec. 2, T13S, R1W) and Swan Lake (Sec. 16, T13S, R1W) in Calhoun County and near Upper Stump Lake (Sec. 33, T7N, R13W) in Jersey County (see Tucker, 1997, for details on these sites). We also colleted turtles from nesting areas near Lower Stump Lake (Sec. 4, T7N, R13W) and Fowler Lake (Sec. 28, T7N, R13W), both also

in Jersey County. All of the nesting areas are interconnected by the Illinois River (Fig. 1).

Egg Collection.—Gravid females were collected at five nesting sites along the Illinois River in Jersey and Calhoun Counties, Illinois. Collections included turtles whose eggs were used in the experiment (=experimental turtles) and turtles whose eggs were not used in the experiment (=reference turtles). We measured plastron length, maximum carapace height, and maximum carapace width (all to 1 mm) and calculated estimated spent body mass (method of Iverson and Smith, 1993) for all females.

Experimental turtles were collected between 30 May and 5 June 1997. Oviposition was induced for experimental turtles on 6 June by intramuscular injection of oxytocin (Ewert and Legler, 1978). The experiment included four clutches each from Upper Stump Lake, Lower Stump Lake, Fowler Lake, and Swan Lake, and five clutches from Pohlman Slough. Reference turtles and their clutches included an additional

44 from Upper Stump Lake, 24 from Fowler Lake, 31 from Pohlman Slough, 32 from Lower Stump Lake, and 226 from Swan Lake. Reference turtles were collected between 28 May and 19 July 1997. Eggs from experimental and reference turtles were patted dry, weighed to the nearest 0.01 g, and uniquely numbered with carbon ink. Prior to their release at the original collecting sites, females were retained for 48 h after oviposition to reduce the frequency of false nesting attempts (Tucker et al., 1995b).

Experimental Procedures.—We prepared four Sterilite brand plastic boxes (58.8 × 40.6 × 15.9 cm) by adding a mixture of 1.11 g water/g vermiculite (water potential: −150 kPa, Packard et al., 1987) to each nest box. Each nest box contained 900 g of vermiculite before adding water. Eggs from each experimental clutch were assigned to each of the four nest boxes using a stratified random design. First, the number of eggs needed for each clutch to be as equally represented in each nest box as possible was determined. Then eggs from the particular clutch were chosen at random for each box until all eggs were assigned.

Hydration was maintained by weekly replacement of the substrate in each nest box with newly mixed substrate of the appropriate kPa. During incubation, experimental eggs were reweighed five times at 12 or 10 d intervals. We determined change in egg mass for this 52 d period by subtracting initial egg mass from the fifth and final egg weight determined on 28 July 1997.

During incubation, all nest boxes were kept at the same vertical height. Nest boxes were horizontally rotated once weekly to spread effects of undetected temperature gradients over all nest boxes. Incubation temperature fluctuated, and was recorded daily with minimum-maximum thermometers. Estimated incubation temperature was near 28 C using the method of Godfrey and Mrosovsky (1994).

Once the first egg pipped, we placed a bottomless waxed paper cup over each egg (Janzen, 1993). We recorded pip date and define incubation period as pip date minus initial date (Gutzke et al., 1984). We then measured hatchling mass (to 0.01 g) and hatchling carapace length (to 0.1 mm). After overwintering in the laboratory, surviving hatchlings were released at the collecting site of the female parent. Mean temperature during the overwintering period (mean 7.8 C, range = 1.2–18.7 C) was monitored with HOBO computer temperature loggers (Onset Computer Corp.).

Statistical Procedures.—Because reproductive traits covary with maternal size at our study area (Tucker and Moll, 1997), we used ANCOVA to compare experimental and reference turtles.

We compared experimental and reference turtles to help preclude the possibility that the experimental turtles were not a representative sample of the turtles nesting at each site. We used estimated spent body mass as the covariate from the four available measures of maternal size (see above) because it was the measure of maternal size that best predicted mean egg mass per clutch using multiple regression.

Statistical analyses of experimental data gathered from eggs and hatchlings were performed with the General Linear Model (GLM) Procedure and the Mixed Procedure in SAS version 6.12 (SAS Institute, 1996). Each set of analyses yield information that cannot be arrived at using one procedure alone (Packard et al., 1999).

The Mixed Procedure estimates variance components using the restricted maximum likelihood method (REML) and Satterthwaite's approximation to correctly compute denominator degrees of freedom (Janzen et al., 1995; SAS Institute, 1996). The mixed procedure correctly estimates covariance parameters for random effects. We examined hatchling mass, hatchling carapace length, incubation period, and change in mass of eggs over incubation (a measure of water-exchange) by designating initial egg mass as the covariate, a fixed effect, and the other variables (i.e., site, nest box, site-nest box interaction, and clutch nested in site) as random effects. Variation due to positioning of nest boxes was spread through all nest boxes by rotating them. Nonetheless, we retain nest box as a random effect in our analyses to remove any remaining variance due to nest box position.

The GLM Procedure was needed to obtain least squares means to assess the magnitude of differences observed among variables. Moreover, the GLM Procedure was required in order to assess the significance levels for the covariance parameters of random effects that are only accurately derivable from the Mixed Procedure (i.e., the method of Packard et al., 1999). The GLM model statement included site, nest box, site-nest box interactions, and clutch nested in site. Each of these variables were also included as random effects with the 'test' option of SAS version 6.12 selected.

Because eggs from the same female in the same nest box were not statistically independent, use of individual eggs as experimental units is tainted by potential effects of pseudoreplication. Pseudoreplication could be avoided by treating the individuals from the same mother and same nest box as a group by using the means for the variables examined as experimental units. Use of means would effectively ignore the random variation within each box and clutch. Moreover, comparisons between this study and the numerous other studies already

published would be complicated by departure from the more widely used method (e.g., Packard and Packard, 1993). We did, however, repeat the analysis using means to investigate the extent to which pseudoreplication might be a factor. These analyses agreed with those we report herein and suggest that pseudoreplication was not an important factor in our analysis using individual eggs as an experimental unit.

We compared means or least squares means (LSM) of experimental variables (i.e., net change in egg mass, incubation period, hatchling mass, and hatchling carapace length) to maternal variables (i.e., plastron length, carapace height, carapace width, and estimated spent body mass) and reproductive variables (i.e., clutch size, mean egg mass per clutch, mean egg width per clutch, and mean egg length per clutch) to investigate possible sources of clutch effects due to maternal identity. We used two sorts of comparisons, stepwise multiple regression and correlation analysis. For multiple regression, experimental variables became dependent variables and maternal and reproductive variables were potential independent variables. We used F-ratios from the first step of each regression to identify independent variables that were significant sources of variance in each dependent variable.

We used Spearman's rank correlation for correlation analysis and partial correlation analysis. For both correlation analysis and ANOVA/ANCOVA, we used the sequential Bonferroni method to identify vales of P that did not exclude the possibility of type I error at 0.05 (Rice, 1989).

RESULTS

Initial Egg Mass.—Neither site ($F_{(4,355)}$ = 2.28, P = 0.0604), status ($F_{(1,355)}$ = 0.51, P = 0.4736), nor their interaction ($F_{(4,355)}$ = 2.32, P = 0.0567) were significant sources of variance in mean egg mass per clutch among the turtles that we studied. In contrast, estimated spent body mass was responsible for nearly all of the variance in mean egg mass per clutch ($F_{(1,355)}$ = 206.15, P < 0.0001) because heavier turtles produced heavier eggs. Nonetheless, the ANCOVA suggested that clutches from experimental turtles were a representative sample overall. Descriptive statistics for all turtles examined are in Table 1.

Initial egg mass for experimental eggs did not differ significantly among the four nest boxes for each site (Kruskal-Wallis test, H < 7.5, P > 0.05, df = 3). Initial mass of experimental eggs, overall, was normally distributed (univariate procedure, P = 0.3466). Initial mass of experimental eggs, uncorrected for differences in maternal size, did vary by site (ANOVA, $F_{(4,270)}$ = 38.84, P < 0.0001, Table 2). Experimental eggs from Pohlman Slough were significantly heavier

TABLE 1. Descriptive statistics for two measures of maternal size, mean egg mass per clutch, and clutch size for all red-eared sliders (*Trachemys scripta elegans*) collected from five nearby sites in west-central Illinois during 1997. N = number of females or number of clutches.

Site-status	N	Plastron length (mm) mean (STD)/range	Spent mass (g) mean (STD)/range	Egg mass (g) Mean (STD)/range	Clutch size (eggs) mean (STD)/range
Swan Lake	230	214 (13)/173–252	1680 (316)/893–2435	10.95 (1.41)/7.12–14.07	12.3 (2.7)/5–19
Pohlman Slough	36	212 (14)/179–233	1565 (328)/890–2276	10.62 (1.51)/7.51–13.72	12.4 (2.9)/6–20
Lower Stump Lake	36	215 (14)/192–250	1697 (379)/1210–2602	10.56 (1.57)/8.23–13.02	14.3 (3.5)/7–26
Upper Stump Lake	48	218 (14)/187–239	1776 (315)/1031–2512	10.89 (1.46)/6.65–14.04	14.0 (2.9)/5–20
Fowler Lake	28	211 (17)/181–250	1620 (384)/993–2589	10.38 (1.96)/7.11–13.37	13.1 (3.1)/8–19

TABLE 2. Means (initial egg mass) and least squares means (other variables) for viable experimental eggs and hatchlings from 21 clutches of red-eared sliders (*Trachemys scripta elegans*) from five nearby sites in west-central Illinois. N = number of eggs or hatchlings. Least squares means are corrected for differences in initial egg mass. Standard errors are in parentheses.

Site	N	Initial egg mass (g)	Change in egg mass (g)	Hatchlings		
				Mass (g)	Carapace length (mm)	Incubation period (days)
Swan Lake	43	10.41 (0.23)	0.73 (0.05)	8.06 (0.05)	30.7 (0.05)	61.1 (0.32)
Pohlman Slough	64	12.69 (0.12)	0.80 (0.04)	8.20 (0.05)	30.8 (0.06)	62.6 (0.25)
Lower Stump Lake	52	10.95 (0.18)	0.84 (0.05)	8.17 (0.04)	30.8 (0.04)	62.6 (0.28)
Upper Stump Lake	60	11.00 (0.10)	0.86 (0.04)	8.20 (0.04)	30.8 (0.04)	62.3 (0.26)
Fowler Lake	56	11.29 (0.06)	1.04 (0.04)	8.24 (0.03)	31.1 (0.04)	63.4 (0.27)

+ Upper Stump Lake   ✳ Fowler Lake   ● Pohlman
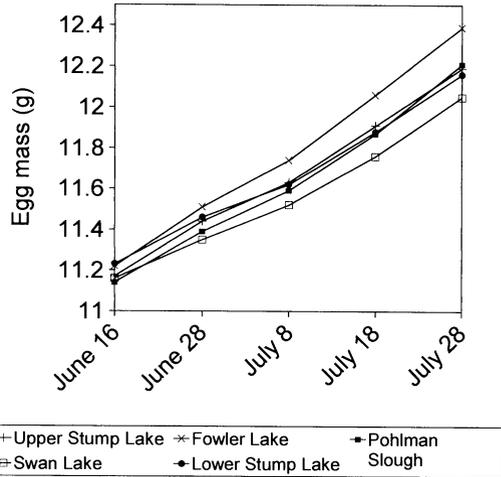⊕ Swan Lake   ◆ Lower Stump Lake   Slough

FIG. 2. Change in egg mass during the first 52 days of incubation for red-eared sliders (*Trachemys scripta elegans*) eggs from five nearby collecting sites.

than eggs from any of the other sites, and remained so even after adjusting for differences in maternal spent body mass using ANCOVA. Experimental eggs from Swan Lake were significantly lighter than eggs from the other four sites, whereas eggs from Upper Stump Lake, Lower Stump Lake, and Fowler Lake were not significantly different. Egg mass for these latter four sites did not differ significantly from each other once adjusted for differences in maternal spent body mass.

Variation in unadjusted initial egg mass may be important because larger eggs may have a greater surface area available for water transport. However, initial egg mass was not significantly correlated with net change in egg mass during incubation (Rho = -0.02, $P = 0.6869$, N = 275). Variation in initial egg mass does not seem to have inordinately affected the response of eggs to incubation environment.

Water-Exchange.—Eggs from all five sites gained mass during incubation (Fig. 2). Clutch effects and initial egg mass accounted for nearly all of the variance in the net change in eggs mass (Table 3). Nonetheless, least squares means did differ significantly among sites (Table 2, Fig. 2) with net change in egg mass during incubation greatest for eggs from Fowler Lake and least for eggs from Swan Lake ($P < 0.0014$ in all pairwise comparisons).

Although least squares means for Fowler Lake were statistically greater than for other sites, overall variation was primarily due to clutch effects. Eggs of some clutches gained more water than did eggs from other clutches. Mean net water gain per clutch for the 21 clutches under study was not correlated with any maternal or

TABLE 3. Variance components for incubation period, net change in egg mass, hatchling mass, and hatchling carapace length. Levels of significance (in parentheses) were determined using 'test' option for random variables in the General Linear Model Procedure of SAS 6.12.

				Hatchlings	
Source of variation	df	Δ egg mass	Incubation period	Wet mass	Carapace length
Site	4	0.000 (0.8101)	0.474 (0.0358)	0.000 (0.3505)	0.006 (0.1329)
Nest box	3	0.000 (0.6546)	0.002 (0.0252)	0.000 (0.1653)	0.000 (0.5641)
Site × Nest box	12	0.001 (0.2585)	0.000 (0.9971)	0.000 (0.9992)	0.000 (0.9642)
Clutch nested in Site	16	0.125 (0.0001)	0.552 (0.0014)	0.012 (0.0001)	0.038 (0.0001)
Residual	239	0.104	3.756	0.056	0.073

reproductive variable. Similarly, none of the potential independent variables used in the multiple regression were significant (Table 4).

However, mean net water gain per clutch was weakly associated with maternal gravid mass if the effect of maternal plastron length was removed by partial correlation (Rho = 0.45, P = 0.046, N = 21 clutches). Apparently, eggs that gained more weight during incubation were more likely to be laid by females that weighed relatively more for their length than those that were relatively lighter for their length.

Hatchling Characteristics.—Nearly all of the variance in hatchling mass and carapace length was due to clutch effects and initial egg mass (Table 3). Larger eggs produced heavier and longer hatchlings.

Although the variance due to site was slight overall (Table 3), pairwise comparisons of hatchlings among sites indicated that hatchlings from Swan Lake (Table 2) were relatively light and were significantly lighter than hatchlings from other sites (P < 0.04 for all pairwise comparisons). In contrast, hatchlings from Fowler Lake had relatively long carapaces that were significantly longer (P < 0.0005 for all pairwise comparisons) than hatchlings from any other site (Table 2).

Although hatchling characteristics did vary by site, the actual difference between extremes was small (Table 2). Overall, mass differed by 0.18 g between hatchlings from Swan Lake and those from Fowler Lake. Hatchling carapace length differed by 0.4 mm between hatchlings from the same two sites.

The basis for the significant clutch effects for both hatchling mass and carapace length could not be definitively identified. Some clutches from all sites produced hatchlings that were both relatively heavy and longer than did other clutches from the same sites. Adjusted means for hatchling mass and carapace length were not associated with any of the maternal variables nor were they associated with gravid mass after

TABLE 4. Summary of F-ratios from the first step of stepwise multiple regression of maternal and reproductive variables (Independent variables) against experimental dependent variables for eggs and hatchlings of red-eared sliders (*Trachemys scripta elegans*) from five nearby sites in west-central Illinois. * P < 0.02 and ** P < 0.003 with 1, 19 degrees of freedom.

	Dependent variables			
Independent variable	Net change in egg mass	Incubation period	Hatchling mass	Hatchling carapace length
Plastron length (mm)	0.50	0.10	3.90	0.06
Carapace height (mm)	1.44	0.00	1.64	1.01
Carapace width (mm)	0.23	0.56	8.00*	0.03
Spent body mass (g)	1.32	0.01	2.54	0.42
Egg mass (g)	0.15	0.01	7.98*	0.09
Clutch size (eggs)	0.08	0.22	0.00	4.15
Egg length (mm)	0.00	0.00	3.40	0.21
Egg width (mm)	0.75	0.05	11.93**	0.07
R^2	0.07	0.03	0.39	0.18

removal of the effect of maternal plastron length. Hatchling mass LSM was, however, associated with mean egg mass per clutch (Rho = 0.62, $P = 0.0028$, N = 21) and with mean egg width per clutch (Rho = 0.72, $P = 0.0002$, N = 21). Mean egg width per clutch was the best potential independent variable in the first step of the multiple regression (Table 4). After variance due to mean egg width per clutch was removed in the second step, mean egg mass per clutch remained significant but maternal carapace width did not. Thus, relatively heavier hatchlings were found among eggs from clutches that had wider and heavier eggs.

In contrast, hatchling carapace length LSM was not associated with mean egg mass per clutch (Rho = -0.07, $P = 0.7737$, N = 21). Instead, hatchling carapace length LSM was associated with clutch size (Rho = 0.53, $P = 0.0142$, N = 21). However, the association between clutch size and hatchling carapace length was weak and not supported by multiple regression (Table 4).

Incubation Period.—Site, clutch effects, and nest box were significant sources of variance for incubation period (Table 3). Nest box effects appeared to be an artifact in this analysis. One nest box had relatively few survivors from Swan Lake and relatively more survivors from Fowler Lake than the others. The incubation period for this box was longer than for the others possibly due to this unbalanced sample. Site effects could be attributed to eggs from Swan Lake and Fowler Lake. Eggs from Swan Lake had a significantly shorter incubation period ($P < 0.005$ for all pairwise comparisons) than eggs from any of the other sites. Eggs from Fowler Lake had a significantly longer incubation period ($P < 0.003$ for all pairwise comparisons) than eggs from any of the other sites.

Incubation period was not associated with any of the maternal or reproductive variables including initial egg size. Furthermore, none of the potential independent variables were a significant source of variance in the multiple regression (Table 4).

DISCUSSION

Our study has important implications for future studies of geographic variation in the physiological response of embryos to incubation environments. Any comparison between geographically distant sites would benefit by inclusion of as many clutches as possible. Because clutch effects are important in all variables that we and others have studied (i.e., Packard, 1991), larger numbers of clutches reduce the possibility that any differences observed between geographically distant regions are due to maternal differences rather than region-specific differences.

We also suggest that any study of geographically distant regions would be strengthened by including turtles from as many local collecting sites for each region as possible. The more sites sampled the less likely it will be that site-specific variation will interfere with evaluation of geographic variation. Sampling a single site per region may be inadequate because any geographic variation found could just as well be due to undetected local site-specific differences.

Maternal effects including those associated with initial egg size and/or maternal identity (i.e., clutch) are important influences on variance in the traits that we studied. Effects of maternal identity resulted in differences in the response of eggs to moisture and in variably sized hatchlings despite removal of variance due to initial egg mass and incubation under similar hydric and thermal environments. Thus, we confirm experimentally the prediction by Janzen et al. (1995) that even if females choose similar hydric environments for their eggs that maternal differences in the response of eggs to moisture would still lead to variably sized offspring.

The biological significance of the site-specific difference that we found is questionable. Except for incubation period, the influence of site was relatively slight compared to those due to clutch or initial egg mass (Table 3). We note, however, that turtles from Fowler Lake, the site whose eggs gained the most water, produced relatively heavy hatchlings with longer carapaces after a longer incubation period than did turtles from other sites despite correcting for initial differences in initial egg size. This finding is consistent with the findings comparing eggs on differing substrate water potentials (Packard, 1991) suggesting that it has biological relevance.

Moreover, the difference in the response of eggs to similar incubation environments that we observed among the five sites is greater than the amount observed between two sympatric species of map turtles (*Graptemys*) (Janzen et al., 1995). However, the differences between extremes for measures of hatchling size for our study are much less than that found by Janzen et al. (1995) between the two species of map turtles, which were collected from the same site.

Our experimental findings may have ecological relevance. Soil types vary among the nesting areas we studied. Swan Lake turtles nest in Oakville loamy fine sand, whereas turtles from the other sites nest in silty loam soil types (Fehrenbacher, 1966; Lilly, 1989). Oakville loamy fine sand is very well-drained and has low available water capacity (Lilly, 1989). In contrast, the silty loam soil types retain water better than Oakville does at Swan Lake (Fehrenbacher, 1966; Lilly,

1989). Because eggs can loose or gain water during incubation depending on water potential (reviewed by Packard, 1991), it may be advantageous for turtles that nest in sandy soil types to lay eggs that respond less rapidly to moisture availability than do the eggs of turtles that nest in silty loam soils. Eggs that take up relatively less moisture during periods with favorable water potentials may also be eggs that loose moisture more slowly during periods when water potential is low and potentially stressful.

The possible sources of maternal effects could not be definitively identified. However, for net change in egg mass and hatchling mass, maternal condition may be implicated. For both of these traits, females or eggs that were heavier than predicted (i.e., high least squares means) are associated with eggs that absorbed the most water or eggs that produced the heaviest hatchlings. It is important to note that effects of egg size and location of collection were removed in these analyses (i.e., Table 3). Although preliminary, our findings are important nonetheless because few studies attempt to identify possible sources of maternal effects despite their general importance (i.e., Packard, 1991; Janzen et al., 1995; Bernardo, 1996; Tucker et al., 1998a). Our preliminary results underscore the importance of collecting data on female parents and placing this information into context with physiological responses of eggs and embryos.

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Cloacal Popping in Snakes

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ABSTRACT.—Tactile stimulation to the Sonoran coral snake (Micruroides euryxanthus) and the western hook-nosed snake (Gyalopion canum) induces an expulsion of air from the cloacal vent which results in an audible popping sound. The behavioral context of cloacal popping differs in these two species, as does the acoustics of the popping sound. The cloacal pops produced by M. euryxanthus are rather consistent and show a low amplitude (50–53.5 dB), limited frequency range (442–5523 Hz), distinct temporal patterning, and harmonics. The cloacal pops released by G. canum are more variable; the initial pops are of high amplitude (70–73 dB) and broad frequency range (359–15,178 Hz), but in subsequent pops the amplitude falls off and the frequency range narrows; no temporal patterning or harmonics were observed. Cloacal popping is driven primarily by the M. Sphincter cloacae but may involve other extrinsic cloacal musculature. The presence of this unusual defensive sound in only these two sympatric snakes suggests its function against a common predator.

With squamate reptiles serving as ecological "model organisms," studies of reptilian ecology, and particularly behavioral ecology, are increasingly popular (Huey et al., 1983; Seigel et al., 1987; Seigel and Collins, 1993). Many aspects of the behavioral ecology of snakes have received little attention from investigators (Greene, 1997). As a group, snakes exhibit a diverse array of