

EMBRYONIC TEMPERATURE INFLUENCES JUVENILE TEMPERATURE CHOICE AND GROWTH RATE IN SNAPPING TURTLES *CHELYDRA SERPENTINA*

SHYRIL O'STEEN*

Department of Ecology and Evolution, University of Chicago, Chicago, IL 60637, USA

*Present address: Department of Ecology and Evolutionary Biology, SH 321, University of California at Irvine, Irvine, CA 92697-2525, USA (e-mail: sosteen@uci.edu)

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Summary

Snapping turtles (*Chelydra serpentina*) demonstrate temperature-dependent sex determination (TSD): intermediate egg incubation temperatures (23–27 °C) produce males, while extreme temperatures produce females. Snapping turtles are also sexually dimorphic: adult males are typically larger than females. Previous researchers hypothesized that male-producing egg temperatures enhanced the growth rate of juvenile turtles, resulting in the adult dimorphism and potentially providing an adaptive benefit for TSD. In reptiles, the choice of ambient temperature can also influence growth. I measured the effect of egg incubation temperature on juvenile growth rate and water temperature choice of *C. serpentina*. Eggs were incubated in the laboratory at 21.5, 24.5, 27.5 or 30.5 °C to produce both sexes, all males, both sexes or all females, respectively. Egg temperature was linearly and negatively correlated with growth rate of both

male and female juveniles. Thus, growth was enhanced, but not maximized, by male-producing egg temperatures. Egg temperature was also negatively correlated with juvenile temperature choice such that, on average, turtles from 21.5 °C eggs selected 28 °C water, while turtles from 30.5 °C eggs chose 24.5 °C water. Additionally, these temperature choices were highly repeatable, even following a 6 month hibernation period at 7 °C. Thus, while male egg temperatures do not directly maximize growth, multiple effects of embryonic temperature may combine to create long-lasting differences in the behavioral physiology of male and female *C. serpentina*. Such differences could be important to the ecology and evolution of TSD.

Key words: temperature preference, growth, development, snapping turtle, *Chelydra serpentina*, temperature-dependent sex determination.

Introduction

Growth rate and choice of environmental temperature can profoundly influence the ecology and fitness of organisms. In reptiles, temperature choice can affect energy requirements, habitat partitioning, locomotor performance and many aspects of development, physiology and behavior (Huey, 1982; Lillywhite, 1987; Rome *et al.* 1992; Peterson *et al.* 1993; Packard and Packard, 1994). In most organisms, growth rate affects life history and can influence total reproductive fitness (Schultz and Warner, 1991; Gotthard *et al.* 1994; Seding *et al.* 1995). Growth rate and temperature choice are highly plastic in most juvenile and adult animals; for example, both traits vary in response to recent ambient temperatures (Cossins and Bowler, 1987; Hutchinson and Dupré, 1992; Rome *et al.* 1992). This fact suggests that early developmental temperatures might influence subsequent growth and temperature choice. However, little is known about the embryonic regulation of these traits. The present study examines such regulation in the common snapping turtle *Chelydra serpentina*.

The consequences of embryonic temperature are particularly germane in reptiles. Reptiles are ectothermic, and both egg-

laying and live-bearing species can experience a range of temperatures during embryogenesis. This temperature variance influences many postnatal traits, including sex (Bull, 1983; Janzen and Paukstis, 1991a; Ewert *et al.* 1994; Lang and Andrews, 1994; Viets *et al.* 1994), behavior (Gutzke and Crews, 1988; Flores *et al.* 1994), locomotor performance and survival (Burger, 1989; Van Damme *et al.* 1992; Janzen, 1995). The effect on sex (temperature-dependent sex determination or TSD) has attracted extensive discussion of its physiological and evolutionary significance. One hypothesis suggests that TSD will co-occur with sexual size dimorphism in reptiles if embryonic temperature determines growth rate in addition to sex (Deeming and Ferguson, 1988, 1989; Janzen and Paukstis, 1991b; Ewert *et al.* 1994). *Chelydra serpentina* is a TSD species with adult males that are larger than females (Gibbons and Lovich, 1990; Ernst *et al.* 1994). In the present study, I examine the hypothesis that male-producing egg temperatures maximize growth rate in *Chelydra serpentina*.

The growth rate of ectotherms depends strongly on ambient temperature. The choice of ambient temperature affects

ectotherm body temperature; body temperature can affect growth by influencing either the rate or efficiency of energy assimilation (Cossins and Bowler, 1987; Sinervo and Adolph, 1989; Rome *et al.* 1992; Avery *et al.* 1993; Beaupre *et al.* 1993a; Elliott *et al.* 1996). Thermal preference and growth rate are also related because both can increase in response to increased feeding (Andrews, 1982; Lang, 1987; Lillywhite, 1987; Hutchinson and Dupré, 1992; Peterson *et al.* 1993). Thus, embryonic effects on juvenile growth could be mediated by juvenile temperature choice. No previous study of turtles has examined the effect of embryonic environment on subsequent temperature choice.

In the present study, I measured the separate effects of embryonic temperature and sex on the growth rate and temperature choice of juvenile *C. serpentina* and examined the interrelationship of choice and growth patterns. Several previous studies examined the effects of egg temperature on turtle growth (Brooks *et al.* 1991; Etchberger, 1993; Bohn and Brooks, 1994; Rhen and Lang, 1995; Roosenburg and Kelley, 1996), but the results of these studies differ, and only one study experimentally separated sex and egg temperature effects (Rhen and Lang, 1995). I separate these effects using a design that complements the previous study, which used hormone treatments to produce both sexes at three egg temperatures (Rhen and Lang, 1995). Snapping turtles eggs are viable across a temperature range of 12°C and produce females at both extremes of the range. I used these traits to produce each sex at three temperatures by incubating eggs at four temperatures that spanned the viable range.

Materials and methods

Egg incubation

Eggs were collected in June of 1992 (three clutches) and 1993 (five clutches) from less than 24 h old nests of *Chelydra serpentina* L. in Whiteside County, Illinois, USA. Eggs were individually numbered, placed in Styrofoam containers with moistened peat moss and transported to the laboratory at the University of Chicago. Eggs were weighed to the nearest 0.1 g, and an equal number (range 4–6) from each clutch was assigned randomly to each of ten (1992) or twelve (1993) plastic boxes (8 cm high × 15 cm wide × 33 cm long) containing moistened vermiculite (−150 kPa ≈ 300 g of dry vermiculite plus 337 g of deionized water) (Janzen *et al.* 1990). Within each box, 18–27 eggs were randomly assigned to a position in a three by nine matrix and half-buried 1–2 cm apart in the substratum.

In the study population, *C. serpentina* eggs are generally viable between 20 and 32°C, produce males between 23 and 27°C, and produce females above 28 and below 21°C (F. Janzen, personal communication). The incubation treatments were designed to detect independent and interactive effects of sex and incubation temperature on subsequent measurements. Four constant-temperature (±0.5°C) incubators were used. The four temperatures, 21.5, 24.5, 27.5 and 30.5°C, were predicted to produce both sexes, all males, both sexes and all females, respectively. In 1992, two egg boxes were placed at 21.5, 24.5

and 30.5°C and four boxes at 27.5°C. In 1993, two boxes were placed at 24.5 and 30.5°C and four boxes at 21.5 and 27.5°C. Box positions were shifted in the incubators daily to mitigate the potential effect of thermal gradients on the developing embryos (Bull *et al.* 1982). To maintain a constant hydric environment, substrata were rehydrated once weekly by adding enough deionized water to return each box, including contents, to its initial mass. The temperatures and hydric conditions used in this experiment are within the range of those measured in natural *C. serpentina* nests (Packard *et al.* 1985). Hatching success was greater than 95% in all treatments.

Hatchling temperature choice and growth rate

On pipping, each egg was isolated in the incubation box by a plastic divider that identified the hatchling. Within 24 h of hatching, turtles were rinsed of vermiculite and adhering membranes, blotted dry and weighed to the nearest 0.1 g. Mid-line carapace length and carapace width at mid-body were measured to the nearest 0.1 mm. Approximately equal numbers of turtles from each of two (1992) or three (1993) clutches were selected at random for the temperature choice and growth experiments. Selected turtles were labeled with an identifying tag visible to a human observer from 1.5 m away. The tags consisted of a labeled 1 cm × 1 cm square of thin plastic, secured with dental floss tied through two needle holes in the marginal scutes of the carapace. Turtles were then returned to their original incubators and housed in the incubation boxes on moistened vermiculite. Hatchlings were not fed while housed in the incubators. A 12 h:12 h light:dark cycle (lights on at 07:00 h) was initiated in each incubator when the first hatchling emerged. This 12 h:12 h cycle was maintained throughout the following experiments. Three to six days after hatching, turtles were released into tanks (described below) where temperature choice and growth rate were monitored for 8–16 weeks. In 1992, all the turtles were released into a single large tank, whereas in 1993 the turtles from different incubation treatments were released in separate groups into a set of smaller replicate tanks. These differences between the years are detailed below.

The 1992 experimental design simulated an isolated natural pond in which hatchlings from nests at different temperatures interact during their first year. Cooler incubation temperatures result in longer incubation times. Thus, the age, size, density and history of hatchlings sharing a pond differs among turtles that experience different incubation temperatures. Similarly, the experimental design combined the effects of incubation temperature with those of the resulting social environment. Turtles were released into one tank at time intervals reflecting the differences in incubation time. Twelve turtles from 30.5°C eggs were released on day 0 (August 14) and removed on day 116. Turtles from 27.5°C eggs were released in two separate groups of twelve, because of the logistic demands of a companion experiment (O'Steen, 1995). The effect of the division into two groups on all subsequent measurements was examined using analysis of variance (ANOVA). Group had no effect, and the 27.5°C turtles were treated as one group for subsequent analyses. These two groups were released on day 6

and day 14, and removed on day 123 and day 130, respectively. Turtles from 24.5 °C were released on day 21 and removed on day 138, and turtles from 21.5 °C eggs were released on day 52 and removed on day 168. The tank was 0.75 m wide \times 3 m long and was filled to 12 cm with tap water. The tank contained three chambers separated by insulated dividers that the turtles could easily surmount, but that prevented water exchange between the chambers. Aquarium heaters and circulating water pumps kept the water in the three chambers at constant temperatures of 20, 25 and 30 \pm 1 °C, respectively. Equal numbers of turtles chosen at random from each treatment group were released into each of the three chambers. Temperature choice was assayed by recording the identity of individual turtles in each chamber once each morning and afternoon, five days a week, every second or third week throughout the experiment. Water temperatures were recorded after each behavior sample using a thermocouple thermometer correct to \pm 0.1 °C. Mean temperature choice was calculated as the mean temperature of the water inhabited by an individual turtle per week and over the full 16 weeks of the experiment. Turtles were fed daily (after the second behavior sample) the amount of Repto-min that they could consume in less than 10 min. Turtles were weighed to the nearest 0.1 g every 2 weeks. Growth rate was calculated as the slope of the regression of $\log_e(\text{mass})$ over time, which approximated a straight line (Sinervo and Adolph, 1989).

The 1993 experiment complemented the 1992 design; 1993 hatchlings were raised in a more artificial but standardized social environment. Groups of 14 turtles from the same egg temperature were raised in replicate tanks. Two tanks were used for each egg temperature which produced a single sex (24.5 and 30.5 °C), and three tanks were used for each mixed-sex incubation temperature (21.5 and 27.5 °C). Tanks were 0.75 m wide \times 0.75 m long, filled to 12 cm with tap water. Each tank contained two chambers with dividers, as in 1992; water in the two chambers was kept at 20 and 30 \pm 1 °C, respectively. The location of individual turtles was recorded twice a day, 5 days a week for 8 weeks following release. Temperature choice was calculated as in 1992. Turtles were fed daily; each tank received 1.5 g of Repto-min for every 100 g of total turtle mass in the tank (calculated using the most recent masses measured). This amount of food was typically consumed in less than 10 min. Turtles were weighed every 2 weeks for 8 weeks. Growth rate was calculated as in the 1992 experiment.

At the end of 8 weeks, the turtle tank groups were moved to a 7 °C room for 6 months (details in O'Steen, 1995). Note that turtle groups entered aestivation on different dates; thus, the relative mass of turtles on particular calendar dates could not be appropriately compared after the start of aestivation. Turtles in all groups lost mass during the 6 months (range 1–10 % of starting mass), a loss similar to that reported for hatchlings recaptured after their first winter in the field (Galbraith *et al.* 1989). In the laboratory, mortality ranging from 0 to 50 % per tank changed the relative number of turtles per tank. Overall mortality was 25 %, much lower than that reported for hatchling *C. serpentina* overwintering in natural nests (Obbard and Brooks, 1981a) and at the low end of annual mortality

estimates for 1- to 4-year-old *Trachemys scripta* turtles (Gibbons and Lovich, 1990). At the end of the 6 month period, the turtles were weighed and returned to the temperature choice tanks. Turtle location was recorded twice daily for 2 weeks, and turtles were fed once daily all the Repto-min they could consume in 10 min; all turtles ate and appeared healthy. After 2 weeks, the experiment was terminated. The experiment was not designed to assay post-hibernation growth rate, and this calculation was not made as this final interval provided only two points for a growth rate regression.

Sexing of turtles

The sex of all turtles from 21.5 and 27.5 °C incubation treatments, 45 turtles from 24.5 °C and 36 turtles from 30.5 °C, was determined by visual inspection of the gonads of dissected animals (Yntema, 1976; Janzen *et al.* 1990). Turtles were killed with an overdose of anesthetic [0.8 ml of 1:1 distilled H₂O:Nembutal (sodium pentobarbital)] injected into the pericardial cavity; they were then dissected and sexed. Dissected turtles were fixed in formalin and preserved in 70 % ethanol. To check the accuracy of the initial sexing procedure, the preserved turtles were sexed separately by F. Janzen and by the author. These latter two assays were conducted blind with respect to incubation temperature; identity tags on the preserved turtles did not identify sex or incubation treatment. Agreement between the three sexing procedures was 100 %. Turtles from 24.5 and 30.5 °C eggs that were not dissected were assumed to be 100 % males and females, respectively, for several reasons. First, all studies of snapping turtle eggs incubated at these and similar temperatures have found 95–100 % single sexes to result (reviewed in Paukstis and Janzen, 1990). Previous studies using the same incubation equipment and population of snapping turtles as the present study found all (25 of 25) 30 °C turtles to be female and all (36 of 36) 26 °C turtles to be male (Janzen, 1995). Finally, in the present study, the 45 dissected turtles from 24.5 °C eggs and 36 turtles from 30.5 °C eggs were all of the predicted sex (males and females, respectively).

Statistical procedures

Data from 1992 and 1993 were analyzed separately because of differences in experimental design. The effects of egg incubation temperature and hatchling sex on hatchling mass, temperature choice and growth rate were examined using separate type III analyses of covariance (ANCOVAs). The type III analyses examine the effect of each variable after removing the effect of all the other variables (Abacus Concepts, 1991). ANCOVAs were generated on a Macintosh computer using SuperANOVA (Abacus Concepts, Berkeley, CA, USA). Egg temperature was tested as a regressor and as a fixed effect in all analyses. Results were equivalent, although treating egg temperature as a regressor always explained a greater portion of the variance. Egg temperature is presented as a regressor for 1992 data, and as a fixed effect for 1993 data, because 1993 tests of tank effects require that incubation temperature be a fixed effect. The other effects included sex, the sex by egg temperature interaction, clutch and egg mass (hatchling mass ANCOVA) or

hatchling mass (temperature choice and growth ANCOVAs) as a covariate. The 1993 temperature choice and growth ANCOVAs include tank effects; tank is nested in incubation temperature (see Table 2). Two *post-hoc* tests were used to define further the relationship between egg temperature and dependent variables. Orthogonal contrasts were used to determine whether the relationship contained linear, quadratic and cubic components (first-, second- and third-order polynomials, respectively). Where needed, Bonferroni/Dunn multiple-comparisons tests were used to compare means among the four egg temperature treatments.

The repeatability of temperature choice was determined by calculating Kendall's coefficient of concordance (*W*) for the egg temperature groups and for individuals within groups (Sokal and Rohlf, 1981). *W* derives from rank orderings and so provides a measure of repeatability relative to the other individuals or groups in the calculation. Weekly means of temperature choice (10 choices per turtle per week) were used for the calculations. In 1992, *W* was calculated across the 16 week monitoring period using every second-weekly mean. In 1993, *W* was calculated over two intervals, first using all eight means from the first 8 weeks after hatching, and then using weeks 7 and 8 and the two weekly means following the 6 month laboratory wintering period.

Results

Sex ratio

Egg incubation temperatures of 21.5 and 27.5 °C produced mixed sex ratios in both years, as predicted. The 21.5 °C incubations produced 18 males and five females in 1992, and 18 males and 23 females in 1993. The 27.5 °C incubations produced 22 males and 32 females in 1992, and 25 males and 15 females in 1993. Sexed turtles from 24.5 and 30.5 °C incubations were all males and all females, respectively. The tables and figures present the sex ratios of turtles used in individual experiments, where appropriate.

Hatchling mass

Within each year, hatchlings from high and low egg incubation temperatures weighed less than hatchlings from intermediate temperatures (Table 1). ANCOVAs for each year indicated that egg temperature, clutch and egg mass explained

the variance for hatchling mass ($P<0.0001$, $P<0.02$, $P<0.0001$, respectively), while sex and the interaction between sex and egg temperature did not ($P>0.26$, $P>0.14$, respectively). Orthogonal contrasts further defined the effect of egg temperature on hatchling mass. These analyses indicated that a quadratic (second-order polynomial) relationship existed in both years ($P<0.0001$) and that a positive linear relationship also existed in 1993 ($P=0.0001$).

Despite these differences among egg temperatures, hatchling mass had no effect on subsequent growth rate or temperature choice (Table 2). Indeed, the pattern of the effect of egg temperature on hatchling mass differed from the pattern for growth rate and temperature choice (see below), and differences in hatchling mass were negligible compared with the effects of growth rate and hatching date on relative juvenile size (see Discussion and Fig. 4).

Hatchling growth rate

Turtles from cooler egg incubation temperatures grew faster than those from warmer egg temperatures in both years of the study (Fig. 1; Table 2). Notably, egg temperature was the only factor that affected growth rate (Table 2), and the temperature effect did not differ between the sexes (Table 2, interaction term). Orthogonal contrasts indicated that the egg temperature effect was linear in both years ($P<0.002$) and had no quadratic ($P>0.34$) or cubic ($P>0.26$) components in either year. Accordingly, the highest average growth rates occurred in the mixed-sex groups of turtles from 21.5 °C eggs, and these rates were not significantly different from those of the all-male groups of turtles from 24.5 °C eggs (Fig. 1; overall mean difference = $0.007\log_e(g)\text{ week}^{-1}$, $P=0.42$, Bonferroni/Dunn).

In 1992, but not in 1993, the separate effects of egg temperature on sex ratio and growth rate created a small difference in the overall average growth rate of the sexes; males grew slightly faster (data from Fig. 1; 1992 mean difference = $0.021\log_e(g)\text{ week}^{-1}$, $t=2.6$, $P=0.01$; 1993 mean difference = $0.005\log_e(g)\text{ week}^{-1}$, $t=0.62$, $P=0.54$). These results derive from the specific egg temperatures used and the number of eggs incubated at each temperature. Thus, if egg temperature affects hatchling growth in nature, any resulting difference between the sexes will depend on the distribution of nest sizes and temperatures.

Table 1. Mass of hatchling snapping turtles incubated at different temperatures

Year	Sex	Egg incubation temperature (°C)							
		21.5	<i>N</i>	24.5	<i>N</i>	27.5	<i>N</i>	30.5	<i>N</i>
1992	Females	10.9±0.36	3			11.3±0.11	27	10.0±0.19	19
	Males	9.9±0.15	17	11.4±0.18	23	11.1±0.20	19		
1993	Females	8.8±0.11	25			9.8±0.08	15	9.4±0.12	34
	Males	9.1±0.13	18	9.8±0.09	36	9.8±0.09	25		

Within each year, hatchlings from intermediate egg temperatures weighed more than hatchlings from extreme temperatures. Statistics are presented in the Results. Units are grams; values are means ± one standard error.

Table 2. ANCOVA results for growth rate and temperature choice of juvenile turtles

Year	Dependent variable	Source of variance	d.f.	<i>F</i>	<i>P</i>
1992	Growth rate	Egg temperature	1	12.44	0.0009
		Sex	1	0.97	0.3304
		Egg temperature \times Sex	1	0.91	0.3459
		Clutch	1	0.06	0.8115
		Hatchling mass	1	2.06	0.1576
		Residual	52		
1992	Temperature choice	Egg temperature	1	15.36	0.0003
		Sex	1	1.83	0.1817
		Egg temperature \times Sex	1	1.70	0.1978
		Clutch	1	5.64	0.0213
		Hatchling mass	1	1.26	0.2678
		Residual	52		
1993	Growth rate	Egg temperature	3	3.86	0.0113
		Sex	1	0.01	0.9160
		Egg temperature \times Sex	1	0.38	0.5405
		Tank (egg temperature)	6	0.18	0.9810
		Clutch	4	1.76	0.1419
		Hatchling mass	1	0.14	0.7128
		Residual	117		
1993	Temperature choice	Egg temperature	3	17.35	0.0001
		Sex	1	0.34	0.5611
		Egg temperature \times Sex	1	0.15	0.6985
		Tank (egg temperature)	6	3.33	0.0046
		Clutch	4	0.43	0.7867
		Hatchling mass	1	0.51	0.4767
		Residual	117		

Egg temperature had a strong and primary effect on both dependent variables.

Hatchling temperature choice

Egg temperature strongly affected the water temperature choice of juvenile turtles. In both sexes, temperature choice was negatively correlated with egg temperature, such that turtles from 21.5 °C eggs selected water 3.5 °C warmer on average than turtles from 30.5 °C eggs (Fig. 2; Table 2). This effect occurred in both years of the study, despite differences in social and physical environments. Orthogonal contrasts confirmed that a linear effect of egg temperature occurred in both years ($P=0.0001$) and also indicated a cubic effect ($P=0.03$) in 1992. These patterns, combined with the number of eggs placed at each temperature and the effects of egg temperature on sex ratio, resulted in juvenile males choosing warmer temperatures overall than females (1992 mean difference 1.2 °C, $t=2.6$, $P=0.01$; 1993 mean difference 0.7 °C, $t=1.4$, $P=0.15$).

Notably, temperature choices were highly consistent among egg temperature groups and among most individuals (Table 3). Turtles even retained their temperature choice ranks after 6 months at 7 °C, despite changing their absolute temperature choices (Table 3; Fig. 3). Among the turtles that survived hibernation, mean selected temperature increased by 1.4 °C (repeated-measures ANCOVA, $F=22.2$, $P=0.0001$), and the increase did not differ between incubation groups or between the sexes ($F=1.0$, $P=0.43$ for incubation group; $F=0.1$, $P=0.99$

for sex). Individuals from only two groups, 1992 turtles from 24.5 °C eggs and 1993 turtles from 21.5 °C eggs, did not show significant temperature choice concordance (W) (Table 3). However, as W derives from rank orderings, this lack of significance may be due to the low variance in temperature choice in these groups (Fig. 3) rather than to the lack of intra-individual repeatability. Low choice variance resulted as all individuals in these groups spent most of their time at the highest water temperature available (data not shown).

Discussion

Effects of egg incubation temperature on hatchling growth

The sexual dimorphism hypothesis suggests that adult male snapping turtles are larger than females in nature because male egg temperatures enhance growth (Deeming and Ferguson, 1988, 1989). The present results suggest that this hypothesis is partly correct, but that multiple factors contribute to size dimorphism. In this study, male egg temperatures enhanced, but did not maximize, growth rates. In the study population, 23–27 °C eggs produce males. Yet the growth rate of turtles from 21.5 °C eggs was not significantly different from that of turtles from 24.5 °C eggs (see Results). Overall, growth rates were linearly and negatively correlated with egg incubation

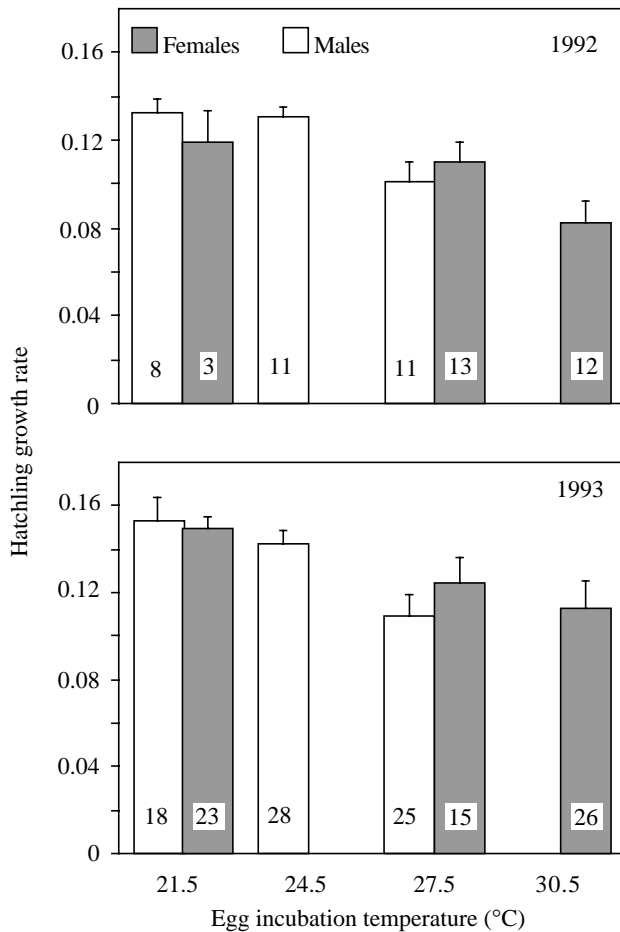


Fig. 1. Egg incubation temperature was negatively correlated with subsequent growth rates of male and female snapping turtles (statistics are given in Table 2 and the Results). Growth rate was calculated as the slope of the regression of $\log_e(\text{mass})$ (in g) over time (in weeks) calculated over the first 16 weeks after hatching in 1992 and the first 8 weeks after hatching in 1993. Values are means \pm S.E.M.; values of N are given within the columns. Different time intervals produced equivalent results (data not shown).

temperatures (Fig. 1, see Results). This result is unique; previous findings are more consistent with the hypothesis. Bobyn and Brooks (1994), Brooks *et al.* (1991) and McKnight and Gutzke (1993) report high growth rates in hatchling *C. serpentina* from eggs incubated at intermediate temperatures (25.5–27 °C) and low growth rates in hatchlings from eggs incubated at extreme temperatures (22 °C and 28.6–31 °C). Rhen and Lang (1995) found that snapping turtles from both 24 and 26.5 °C eggs grew faster than turtles from 29 °C eggs. Rhen and Lang (1995) did not study an extreme low egg temperature, and so their results are consistent both with mine and with the sexual dimorphism hypothesis.

One factor, access to a thermal gradient, may explain the different results of these studies. Snapping turtles displaying a curvilinear relationship between egg temperature and growth rate were raised at uniform temperatures (21–25 °C) (Brooks *et al.* 1991; McKnight and Gutzke, 1993; Bobyn and Brooks,

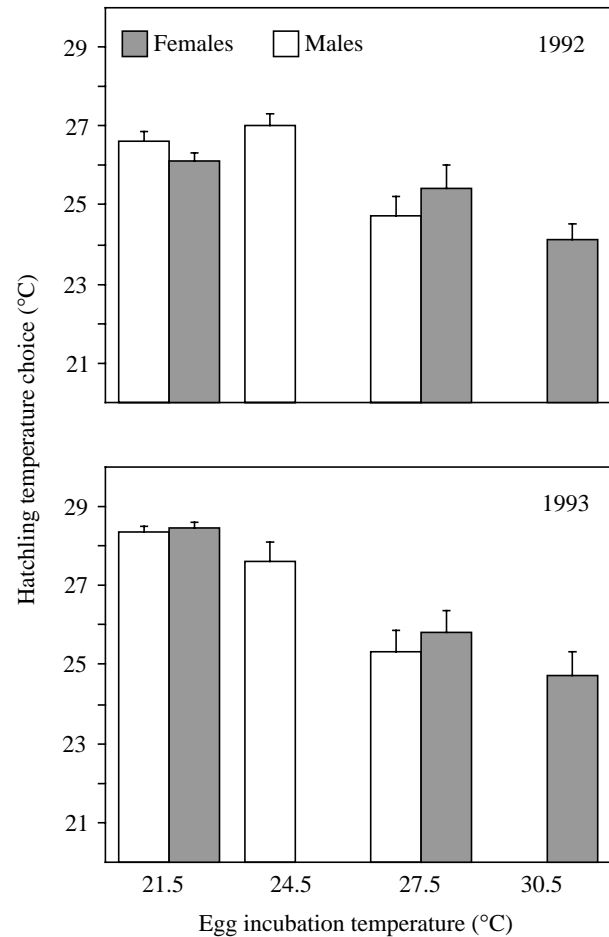


Fig. 2. Increasing egg incubation temperatures resulted in decreasing water temperature choice among juvenile turtles of both sexes. Water temperature is highly positively correlated with body temperature in field studies of snapping turtles (Punzo, 1975; Obbard and Brooks, 1981b), and temperature choice is assumed to reflect body temperature here. Temperature choice was averaged over the first 16 and 8 weeks after hatching in 1992 and 1993, respectively. Statistics are presented in Table 2 and the Results. Sample sizes are as in Fig. 1. Values are means \pm S.E.M.

1994). Turtles showing linear or potentially linear relationships between growth rate and egg temperature had access to thermal gradients of at least 21–30 °C (this study, Rhen and Lang, 1995). Thus, high growth rates of juvenile *C. serpentina* from low egg temperatures (below 24 °C) may depend on the ability to select high temperatures behaviorally. This conclusion is supported by many studies that find differing growth rates between reptiles in thermal gradients and those in constant-temperature environments (e.g. Lang, 1987; Sinervo and Adolph, 1989; Denver and Licht, 1991). Turtles inhabit thermal gradients in nature; thus, hatchlings from cool nests may have maximal growth rates if warm temperatures are available.

Interestingly, negative correlations between growth rate and egg temperature are not reported in other turtles. The diamondback terrapin (*Malaclemys terrapin*) displays a positive correlation between egg temperature and hatchling growth rate (Roosenburg and Johnson, 1995; Roosenburg and

Table 3. Kendall's coefficients of concordance (W) for the weekly mean temperature choice of individuals and groups

	Year and time		
	1992	1993	1993
	16 weeks	8 weeks	36 weeks
	(N=8)	(N=8)	(N=4)
	Before winter		Across winter
Individuals within egg temperature groups			
21.5 °C	0.29***	0.15	0.34
24.5 °C	0.19	0.64***	0.44*
27.5 °C	0.49***	0.53***	0.57***
30.5 °C	0.53***	0.68***	0.65***
Egg temperature groups	0.71*	0.96***	1.00***

W can take values from 0 to 1; 1 indicates perfect concordance over time.
Time is the real time spanned by the measurements.
N is the number of weekly means used to calculate W.
* $P < 0.025$, *** $P < 0.001$.

Kelley, 1996), while the loggerhead musk turtle (*Sternotherus minor*) displays no correlation (Etchberger, 1993). These studies did not specify sex effects or juvenile thermal environments; these factors may help to explain the divergent patterns. Divergent patterns also appear in other reptiles. In crocodilian studies, for example, Joanen *et al.* (1987) report that extreme egg temperatures reduce growth rate in juvenile *Alligator mississippiensis* raised in constant-temperature environments, yet Webb and Cooper-Preston (1989) report a positive correlation between egg temperature and growth rate of juvenile *Crocodylus porosus* raised in a thermal gradient. Clearly, detecting any broad patterns of relationship between embryonic temperature and juvenile growth rate in reptiles may require allowing and measuring juvenile temperature choice.

Another factor that may affect juvenile size dimorphism in reptiles is hatching date. Egg incubation temperature affects incubation time; the warmest eggs hatch first (e.g. Yntema, 1978). In the present study, the late hatching date of turtles from 21.5 °C eggs caused these animals to be smaller than the others during most of the study, despite high growth rates (Fig. 4). In contrast, hatching-date-induced size differences among the other turtles disappeared by the time the natural population typically enters hibernation (approximately day 70), and size thereafter diverged according to relative growth rates (Fig. 4). As a result of these patterns, turtles from 24.5 °C eggs may stay larger than turtles from 21.5 °C eggs for several seasons and may be larger than turtles from warmer eggs in adulthood. This possibility awaits testing; it was beyond the scope of the present study to determine whether early size or growth rate differences persist beyond the first hibernation (see Materials and methods).

To affect adult dimorphism, juvenile size or growth differences would need to persist through maturity, which in snapping turtles takes 4–19 years (Ernst *et al.* 1994).

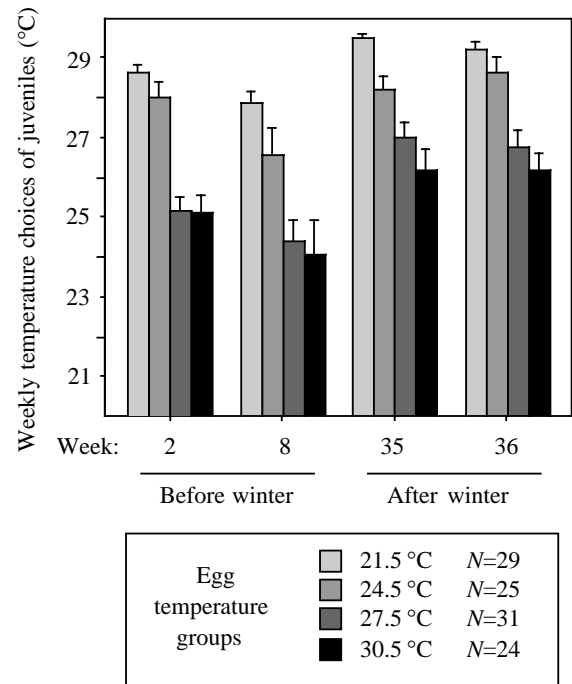


Fig. 3. The effects of egg incubation temperature on juvenile temperature choice persisted over time. Turtles maintained consistent relative temperature choices before and after the laboratory wintering period, despite increasing their absolute temperature choices after the winter (Results, see Table 3 for statistics). Weekly temperature choices represent the means of 10 choices per turtle per week. Data from females and males did not differ significantly; sexes are combined in the graph. The data include only 1993 turtles that survived overwintering. Data from 1992 showed similar consistency (Table 3). Values are means + S.E.M.

Alternatively, or additionally, egg temperature could affect adult dimorphism by influencing the differential persistence of growth rates. In a study of natural populations in Iowa, male and female hatchling snapping turtles grew at similar average rates (Christiansen and Burken, 1979), as was found in the present study (see Results). However, in nature, female growth rates declined before male rates, such that males grew faster than females for turtles aged 5–7 years (Christiansen and Burken, 1979). The physiological and ecological mechanisms that create adult sexual size dimorphism in snapping turtles may be fascinatingly complex and remain incompletely known.

Nonetheless, the present study identified physiological and behavioral factors that can contribute to sexual size dimorphism among juvenile *C. serpentina*. The negative correlations between egg temperature and juvenile growth rate, and between egg temperature and juvenile temperature choice (also see below), could create dimorphism when combined with patterns of availability and choice of nest temperatures in a natural population. For example, if nest temperatures below 24 °C were either rarely available or rarely selected by females, then the resulting male hatchlings might, on average, select warmer water and grow faster than females. This scenario is of interest because it allows behavioral mediation of sexual

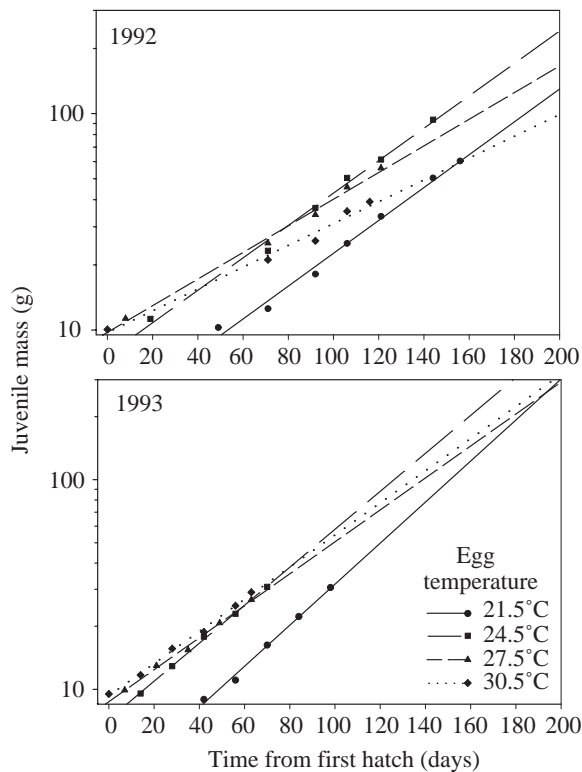


Fig. 4. Hatching date affected the relative mass of juvenile turtles. Turtles from 30.5°C eggs hatched first, in early August. Higher growth rates of turtles from later-hatching, cooler eggs resulted in size convergence of turtles from 24.5, 27.5 and 30.5°C eggs near the date that the natural population typically enters hibernation (late October, day 70) (Obbard and Brooks, 1981b; Meeks and Ultsch, 1990). However, the extremely late hatching date of 21.5°C eggs left these turtles smaller at day 70. The relative mass of the different groups could change as depicted by the extrapolated lines if relative growth rates persisted after hibernation (not tested here). The slopes of the regression lines equal the growth rates presented in Fig. 1. Points represent means for each measurement date, with samples sizes as in Fig. 1.

size dimorphism within a population. Both the nest site choice by females and the temperature choice of hatchlings could mediate the relative growth rates of the sexes. The effect of natural nest temperatures on juvenile growth rates and temperature choice is the subject of a current study.

Effects of egg incubation temperature on hatchling temperature choice

In this study, egg temperature had a strong and long-lasting influence on the temperature choice of juvenile snapping turtles, and this influence was the same in both sexes. This study is the first to report on this relationship in turtles. Two previous studies of reptiles examined this relationship, and they suggest that embryonic temperature does not necessarily dictate temperature choice. In live-bearing *Thamnophis elegans* garter snakes, maternal temperature during pregnancy does not affect juvenile temperature selection (Arnold *et al.* 1995). In contrast, Lang (1987) found that either egg temperature or sex was correlated with temperature choice in *Crocodylus siamensis*, a TSD

species. Four male hatchlings from 33°C eggs selected temperatures on average 3.3°C higher than two females from 28°C eggs (Lang, 1987). In the present study, male snapping turtles also selected higher temperatures on average than did females. Interestingly, egg temperature is positively correlated with temperature choice in crocodiles, but negatively correlated in snapping turtles. Males choose higher temperatures in both species because egg temperature has opposite effects on sex in the two species. Thus, either the mechanistic relationship between egg temperature and temperature choice is different in crocodiles and snapping turtles or, in crocodiles, sex has a predominant influence on temperature choice.

Tank group also affected temperature choice in *C. serpentina* (Table 2). In 1993, turtles were grouped by egg temperature and the groups were raised in replicate tanks (see Materials and methods). The tank effect indicates that either the social groups or the physical traits of the different tanks influenced temperature choice. The former is more likely, as all the tanks had the same physical structures and water temperature profiles, while social factors are known to affect thermoregulatory behavior in a number of ectotherms (reviewed in Lang, 1987). I did not test for social effects experimentally, but several factors indicate that the influence of egg temperature on hatchling temperature choice is robust to social and abiotic effects. First, incubation temperature effects are highly significant even when tank effect is controlled for (Table 2). Second, egg temperature was negatively correlated with hatchling temperature choice in both 1992 and 1993, despite differences in the social and physical environments. Finally, the influence of egg temperature on hatchling temperature choice remained after overwinter mortality had changed the size and composition of social groups (Fig. 3; Table 3).

The ANCOVA also indicated that temperature choice varies between clutches (Table 2). Clutch effects occurred only in 1992, yet they show that maternal or genetic factors can influence juvenile turtle temperature choice. This study did not include sufficient clutches to quantify genetic relationships. However, the availability and large size of *C. serpentina* clutches lend them to research on genetic correlations and genotype by environment interactions (Janzen, 1992; Rhen and Lang, 1995). The potentially broad importance of temperature choice could make such studies worthwhile.

Relationship between temperature choice and growth rate

Egg temperature had similar effects on growth rate and temperature choice in the present study. This result is unsurprising in that growth and temperature choice are interrelated in reptiles; selecting higher temperatures can increase growth rate, and increased feeding can increase both growth rate and temperature choice (Lang, 1987; Lillywhite, 1987; Rome *et al.* 1992; Avery *et al.* 1993; Beaupre *et al.* 1993a). The present study did not separate feeding, growth and temperature choice experimentally. However, several points suggest that the primary effect of egg temperature was on temperature choice and that choice mediated growth rate. First, as discussed earlier, the growth rate of *C. serpentina* is not a

necessary function of egg temperature; instead, egg temperature effects can be mediated by access to a thermal gradient.

Second, feeding does not increase the temperature chosen by snapping turtles. A laboratory study of juveniles found a negative effect (Knight *et al.* 1990), and a study of adults in the wild found no effect, of feeding on temperature choices of *C. serpentina* (Brown and Brooks, 1991). Additionally, feeding rate does not necessarily dictate growth rate. In the second year of the present study, I limited food to a proportion of the total turtle mass in a given tank, recalculated every 2 weeks. Initial turtle masses did not differ across tanks and treatments, and the food was all consumed. Thus, turtles from all incubation temperatures initially consumed similar amounts of food, yet growth rates still diverged.

Finally, ANCOVA supports the prediction that the effects of egg temperature on growth rate result from their effects on temperature choice. ANCOVAs for growth and choice were constructed using the reciprocal traits as covariates (Table 4). The results show that egg temperature affected temperature choice even when the effects of growth rate on choice were removed. In contrast, growth rate was not affected by egg temperature when the effects of temperature choice on growth rate were removed (Table 4). These analyses obviously do not determine cause and effect, but are nonetheless consistent with the prediction.

Importance of temperature choice

Embryonic effects on subsequent temperature choice may be

broadly important as, in addition to growth, these effects could impact on a variety of physiological, ecological and evolutionary factors. This potential importance is strengthened by the repeatability of relative temperature choices. Both individuals and groups of turtles maintained stable relative choices throughout this study (Table 3; Fig. 3). Notably, turtles maintained their relative choices even while increasing their absolute temperature choices after hibernation. If such radical temperature changes in the juvenile environment do not override the effects of embryonic temperature, then differences in temperature choice may persist well beyond the life stages assayed here. In other reptiles, egg temperatures can permanently affect several traits, including sex (Bull, 1987) and aggressive and reproductive behaviors (Gutzke and Crews, 1988; Flores *et al.* 1994) and have long-lasting effects on other traits including growth rate (Joanen *et al.* 1987; Webb and Cooper-Preston, 1989) and locomotor behaviors (Burger, 1989). In contrast, the effects of temperature acclimation during later life stages are typically reversible. For example, thermal acclimation of critical thermal maxima can be reversed in 3 weeks in juvenile *C. serpentina* (Williamson *et al.* 1989), and acclimation of thermal preferences is typically reversible within several months in many adult reptiles (Lillywhite, 1987; Tsuji, 1988; Rome *et al.* 1992). The present study indicates that, in *C. serpentina*, embryonic effects on temperature choice may persist much longer than, and in spite of, the effects of temperature on later life stages.

Long-lasting embryonic effects on temperature choice could impact broadly on turtle physiology, ecology and fitness if

Table 4. ANCOVAs of growth rate and temperature choice, including these traits as covariates

Year	Dependent	Source of variance	d.f.	F	P
1992	Growth rate	Egg temperature	1	0.87	0.3544
		*Temperature choice	1	65.46	0.0001
		Clutch	1	3.67	0.0606
		Residual	54		
1992	Temperature choice	Egg temperature	1	4.48	0.0388
		*Growth rate	1	65.46	0.0001
		Clutch	1	11.39	0.0014
		Residual	54		
1993	Growth rate	Egg temperature	3	1.91	0.1312
		*Temperature choice	1	150.16	0.0001
		Tank (egg temperature)	6	2.67	0.0184
		Clutch	4	4.62	0.0017
		Residual	119		
1993	Temperature choice	Egg temperature	3	16.13	0.0001
		*Growth rate	1	150.16	0.0001
		Tank (egg temperature)	6	6.15	0.0001
		Clutch	4	2.59	0.0400
		Residual	119		

The analyses suggest that growth rate is primarily dependent on hatchling temperature choice and not on egg temperature, as egg temperature does not explain growth rate variation when temperature choice is used as a covariate. Egg temperature does explain temperature choice variation when growth is used as a covariate.

*Indicates the covariate. To simplify presentation, analyses were reduced to significant effects.

Full ANCOVAs as in Table 2 give the same results.

these effects occur in nature. In addition to affecting growth, temperature choice might affect energy requirements, locomotor performance, habitat choice and other traits (Huey, 1982; Lang, 1987; Lillywhite, 1987; Rome *et al.* 1992; Beaupre *et al.* 1993b; Peterson *et al.* 1993; Sinervo and Adolph, 1994). Furthermore, the distribution of nest sizes and temperatures could result in male and female turtles that differ in these traits. For example, males from cooler nests might occupy the warmer shallow edges of a pond, while females from warmer nests might occupy cooler deeper water, thus exposing the sexes to differential predation risks, food sources, retreat and hibernation sites. If found, such sex differences would support an adaptive basis for the evolution of TSD (Bull, 1983, 1989; Deeming and Ferguson, 1989; Janzen and Paukstis, 1991b; Ewert *et al.* 1994; Roosenburg, 1996).

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