



Temperature during embryonic and juvenile development influences growth in hatchling snapping turtles, *Chelydra serpentina*

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Received 25 July 1998; accepted 24 September 1998

Abstract

Embryonic temperature influenced subsequent growth in juvenile snapping turtles, *Chelydra serpentina*: incubation temperatures of 24 and 26.5°C enhanced growth relative to a temperature of 29°C. Although embryonic temperature normally determines gonadal sex in this species, experimental manipulations revealed that temperature effects on growth were independent of sex. Ambient temperature also affected growth: juvenile turtles grew slowly in a cool (19°C) versus a warm (28°C) environment. In a parallel experiment, turtles from different embryonic temperatures displayed different patterns of temperature choice in response to nutritional status or time of day. We tentatively conclude that embryonic temperature has both direct and indirect (i.e., through temperature choice) effects on growth in snapping turtles. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Chelydra serpentina*; Growth; Common snapping turtle; Temperature-dependent sex determination; Behavioral thermoregulation

1. Introduction

Ambient temperature influences the biology of all organisms, but such effects are especially pronounced in ectotherms. Temperature during embryonic development, for instance, has permanent phenotypic effects on many reptiles. Incubation temperature determines gonadal sex in some lizards, many turtles, and all crocodilians examined to date (reviewed in Ewert et al., 1994; Lang and Andrews, 1994; Viets et al., 1994). While progress has been made with respect to the developmental mechanism of temperature-dependent sex determination (TSD) (Pieau, 1996; Crews, 1996; Lance, 1997), little is known about how temperature affects the developmental mechanisms controlling other

traits. In this category are the effects of incubation temperature on hatchling size (Packard et al., 1987, 1988; Allsteadt and Lang, 1994; Spotila et al., 1994; Rhen and Lang, 1998a), body pattern and pigmentation (Murray et al. 1990; Etchberger et al., 1993), morphology (Arnold, 1988), behavioral thermoregulation (Lang, 1987; O'Steen, 1998), post-hatching growth (Joanen et al., 1987; Brooks et al., 1991; McKnight and Gutzke, 1993; Boby and Brooks, 1994; Rhen and Lang, 1995; O'Steen, 1998), and physiology and behavior (Gutzke and Crews, 1988; Burger, 1990, 1991; Flores et al., 1994; Tousignant and Crews, 1994; Shine, 1995; Shine et al., 1997).

Embryonic temperature effects, however, are fairly well studied in regard to sex determination and juvenile growth in the common snapping turtle, *Chelydra serpentina*. Previous work has documented strong incubation temperature effects on post-hatching growth (Brooks et al., 1991; McKnight and Gutzke, 1993; Boby and Brooks, 1994; O'Steen, 1998). Specifically, low and high temperatures produced slow growing tur-

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gles, relative to turtles from intermediate temperatures. Yet, temperature and sex effects on growth were confounded in these studies because incubation temperature also determines gonadal sex; low and high temperatures produce females whereas intermediate temperatures produce males in all populations of snapping turtles studied to date (Yntema, 1979; Wilhoft et al., 1983; Ewert et al., 1994; Janzen, 1992; Bobyn and Brooks, 1994; O'Steen, 1998; Rhen and Lang, 1998b; J.W. Lang, unpublished data). Rhen and Lang (1995) experimentally separated these normally confounded effects and found that incubation temperature influenced growth whereas gonadal sex did not. This temperature-induced association between sex and growth may be adaptive if enhanced growth in snapping turtles from intermediate temperatures has differential effects on male vs. female fitness (Charnov and Bull, 1977; Conover and Heins, 1987; Rhen and Lang, 1995). Such phenotypic plasticity is generally thought to be an adaptive response to environmental variation (Via et al., 1995). Notwithstanding evolutionary explanations, the mechanism(s) by which embryonic temperature affects growth in snapping turtles has not yet been elucidated.

Based on the finding that embryonic temperature also affects behavioral thermoregulation, O'Steen (1998) suggested that incubation temperature indirectly influences juvenile growth in the snapping turtle. Specifically, it was posited that incubation temperature affects temperature choice which in turn influences growth. This hypothesis is plausible because body temperature, and thus temperature choice, profoundly influences growth rates and other physiological processes in many reptiles (Huey, 1982; Arnold and Peterson, 1989; van Marken Lichtenbelt, 1992; Sinervo and Adolph, 1994; Avery, 1994; Autumn and De Nardo, 1995; Dorcas et al., 1997). However, such a scenario is just one explanation for incubation temperature effects on growth in young snapping turtles. Alternatively, incubation temperature may have direct effects on growth potential independent of its effects on temperature choice. Finally, it is possible that both mechanisms contribute to variation in growth. To investigate the extent to which incubation temperature, gonadal sex, and juvenile temperature influence growth in young snapping turtles, we limited the potential for behavioral thermoregulation, thereby uncoupling the confounding effects of incubation temperature on hatchling growth and temperature choice. We also determined the effects of incubation temperature on temperature choice in a thermal gradient.

2. Materials and methods

Turtles used in this study were a random subsample of four clutches from another experiment (Rhen and Lang, 1994, 1995). In all, twenty clutches of snapping turtle eggs were collected within 24 h of oviposition in and around Lake Itasca State Park, Clearwater County, Minnesota (USA) in early June, 1992. Eggs were kept for 4–7 days at 16–18°C to slow embryonic development until eggs were transported to the University of North Dakota for incubation. Eggs were weighed to within 0.1 g and candled for viability, and infertile eggs were removed. Approximately equal numbers of viable eggs from each clutch were randomly assigned to one of twelve treatment groups that consisted of one of three constant incubation temperatures and one of four hormone manipulations.

Eggs were incubated at 24, 26.5, or $29 \pm 0.1^\circ\text{C}$, in vermiculite-filled plastic containers within foam box incubators, as described in Lang et al. (1989). These temperatures are symmetrically distributed across the viable range of constant temperatures for embryonic development in this species. In this population, incubation temperatures of 24 and 26.5°C normally produce only males while 29°C produces mostly females. Hormone manipulations reversed this pattern of sex determination (see Rhen and Lang, 1994). Briefly, the vehicle control (5 μl ethanol) had no effect on sex ratio versus non-treated controls at any of the incubation temperatures. In contrast, treatment of eggs with estradiol-17 β (15 μg dissolved in 5 μl ethanol) produced females at all three temperatures. Sex ratio was reduced from 99.6% male in control (i.e., non-treated and ethanol-treated) turtles to 6% male in estradiol-17 β treated turtles that were incubated at 24 and 26.5°C. Similarly, sex ratio was reduced from 24.6% male in control turtles to 0% male in estradiol-17 β treated turtles that were incubated at 29°C. Conversely, the aromatase inhibitor fadrozole (100 $\mu\text{g}/5 \mu\text{l}$ ethanol; CGS 16949A; Ciba Geigy) produced significantly more males (i.e., 68% male) at 29°C but had no effect on sex ratio at the male-producing temperatures of 24 and 26.5°C. In this way, we were able to control for the normally confounded effects of incubation temperature and gonadal sex. Sex-reversed males and females appear normal because they had sex-typical gonadal histology, plasma sex steroid levels, initial total mass, residual yolk mass, fat body mass, and growth in thermal gradients (Rhen and Lang, 1994, 1995; Rhen et al., 1996; Rhen and Lang, 1998a).

While ten of the original 20 clutches were sacrificed at a young age to determine temperature and sex effects on hatchling traits (Rhen and Lang, 1998a), growth was monitored in the other ten clutches for approximately 6 months within thermal gradients (Rhen and Lang, 1995). After this initial experiment in which

turtles were allowed to thermoregulate, we picked a random sample of four clutches (total $N = 176$) to determine the relative importance of incubation temperature, gonadal sex, and juvenile temperature effects on growth and to determine incubation temperature effects on behavioral thermoregulation. To accomplish this, approximately two thirds of the turtles from each clutch, representing each incubation temperature ($N = 39$ from 24°C ; $N = 40$ from 26.5°C ; $N = 40$ from 29°C), were randomly assigned to one of two constant low temperature ($19 \pm 0.2^{\circ}\text{C}$) or one of two constant high temperature ($28 \pm 0.1^{\circ}\text{C}$) pools. Turtles were weighed three times: once at the start of the experiment (i.e., at day 0) and twice thereafter (i.e., at days 33 and 76).

Growth rates were determined using the following formula, which is adapted from Rhen and Lang (1995). Individual masses at each measurement period were transformed to their natural logarithm and growth rates for each turtle were calculated using the following formula where $N =$ measurement number:

Specific Growth Rate =

$$\left(\frac{1}{2} \sum \ln \text{Mass}_N - \ln \text{Mass}_{N-1} / \text{Age}_N - \text{Age}_{N-1} \right) \times 1000$$

Thus, specific growth rates were averaged over two time periods: from 0–33 days and 33–76 days.

The remaining third of the turtles were held in one of the original thermal gradients to monitor their temperature choice: there were 20 turtles each from incubation temperatures of 24 and 26.5°C , and 17 turtles from an incubation temperature of 29°C . Incubation temperature was identified with color coded beads attached to the rear margin of the turtles' shells. The gradient was partitioned into two thermally distinct sections using bricks and turtles moved freely between the two sections during the entire experiment. The average temperature was $21.8 \pm 0.1^{\circ}\text{C}$ (similar to the constant low temperature holding pools) throughout the cool section of the gradient. The warm section had an average temperature of $23.8 \pm 0.1^{\circ}\text{C}$ in its coolest portion and an average of $27.2 \pm 0.4^{\circ}\text{C}$ (similar to the constant high temperature holding pools) in its warmest portion. We recorded the number of turtles from each incubation temperature that were in the warm and cool sections of the gradient five times daily at 0800, 1000, 1200, 1500, and 1700 h, for 23 days. Temperatures within the pool were also monitored at these times. Thus, we used the proportion of turtles in the warm section of the gradient as an index of thermoregulatory behavior for each incubation temperature. Such proportions have been used to measure basking behavior in the snapping turtle and other rep-

tiles (Rand, 1967; Huey, 1974; Obbard and Brooks, 1979; Hertz, 1981).

Following the protocol of Rhen and Lang (1995), turtles in both the thermoregulation and constant temperature experiments were fed excess ground frozen smelt every other day at 1200 h for the duration of the experiment. Food was spread throughout the pools to minimize any social interactions that could have excluded individuals from access to food (Froese and Burghardt, 1974; Harless, 1979; Bjorndal, 1986; McKnight and Gutzke, 1993). Photoperiod was 12L:12D with the photophase beginning at 0700 h for turtles in both experiments.

Version 2 of JMP for Apple Macintosh was used for all statistical procedures (SAS Institute, 1989). Growth rates in the constant temperature experiment were analyzed using ANCOVA. Clutch identity, incubation temperature, hormone manipulation (including the vehicle-treated control), and their interaction effects were independent variables. The third order interaction among these variables was not included in the model because some of the cells in the design matrix were empty due to limited sample size. Effects that included clutch identity were designated random variables in a mixed model design. Hatchling sex was also used as an independent variable but no interaction terms with sex were included, again because some cells in the design matrix were empty. Juvenile temperature (i.e., cool or warm constant temperature) and its interaction with incubation temperature were also used as independent variables. Finally, we used growth rate from the initial experiment (i.e., when turtles could thermoregulate) as a covariate.

Temperature choice (i.e., number of turtles in the cool vs. warm sections of the gradient) was analyzed using a logistic model (Sokal and Rohlf, 1981). Since body temperature rapidly equilibrates with ambient water temperature in juvenile snapping turtles and other aquatic reptiles (Rhen and Lang, unpublished data; Brown and Brooks, 1991; Manning and Grigg, 1997; Seebacher and Grigg, 1997) and body temperature is strongly correlated with ambient air temperature in basking snapping turtles (Obbard and Brooks, 1979), the proportion of turtles in the warm section of the gradient is a good index of relative body temperatures. We did not determine the identity of individual turtles when counting turtles from each temperature within each section of the gradient. Thus, we only use incubation temperature, time after feeding, and their interaction as independent variables in the analysis of temperature choice. Gonadal sex, hormone treatment, and clutch were not used as factors in this analysis. We first tested whether temperature choice was influenced by incubation temperature, time after feeding, and their interaction. We then tested the specific null hypothesis of no temperature choice for turtles from

each incubation temperature. This hypothesis predicts an equal proportion of turtles will be found within the cool and warm sections of the gradient because these sections were equal in area. The alternative hypothesis of temperature choice predicts that a disproportionate number of turtles (i.e., greater or less than 50%) will be found in the warm or cool sections of the gradient.

3. Results

Embryonic incubation temperature significantly affected growth rate, even after turtles were transferred to constant temperature holding conditions (Table 1). Moreover, incubation temperature effects were still evident when statistically controlling for previous growth rate in a thermal gradient; the covariate was highly significant (Table 1). Juvenile temperature also affected growth rate (Table 1): a warm ambient temperature enhanced growth relative to cool ambient temperature. There was no detectable interaction between incubation temperature and juvenile temperature. Thus, turtles from 24 and 26.5°C grew faster than turtles from 29°C in both the cool and warm environments (Fig. 1). No other independent variables had detectable effects on juvenile growth.

Incubation temperature significantly affected the proportion of turtles in the warm section of the thermal gradient (Fig. 2; Likelihood Ratio Chi-square or $LR\chi^2 = 56.4$, $d.f. = 2$, $p < 0.0001$). Overall, a greater proportion of turtles from 26.5°C were in the warm section of the gradient than would be expected by chance (i.e., greater than 50%). A slightly smaller proportion of turtles from 24°C were in the warm section, but there were still more than would be expected by chance alone (i.e., greater than 50%). In contrast,

equal proportions of turtles from 29°C were in the warm and cool sections of the gradient. Time after feeding had a significant effect on the proportion of turtles in the warm section of the gradient ($LR\chi^2 = 11.6$, $d.f. = 9$, $p = 0.0007$). There was also a significant interaction between incubation temperature and time after feeding ($LR\chi^2 = 8.0$, $d.f. = 18$, $p = 0.018$). More turtles from an incubation temperature of 24°C moved into the warm section after feeding and then gradually moved out of this section over the next 48 h, until the next feeding (Fig. 2; $LR\chi^2 = 42.7$, $d.f. = 9$, $p < 0.0001$). In contrast, there was no detectable change over time in the proportion of turtles from an incubation temperature of 26.5°C that were in the warm section of the gradient (Fig. 2; $LR\chi^2 = 6.7$, $d.f. = 9$, $p = 0.67$). Finally, turtles from an incubation temperature of 29°C tended to move into the warm section over the course of each day from 0800 to 1700 h, regardless of time after feeding (Fig. 2; time of day $LR\chi^2 = 9.7$, $d.f. = 4$, $p = 0.05$).

4. Discussion

Our results show that embryonic temperature influences growth in juvenile snapping turtles that were held in disparate, constant thermal environments. Incubation temperature effects on subsequent growth at constant ambient temperatures were similar to incubation temperature effects on growth in a thermal gradient: turtles from male-producing temperatures grow faster than turtles from a female-producing temperature (this study; Rhen and Lang, 1995). Although the temperature effect in the current study does not meet the usual criterion for significance (i.e., $p < 0.05$), we believe that this effect is of biological significance for a

Table 1
Summary of the mixed model analysis of covariance (type III sums of squares) for growth rate of hatchling snapping turtles

Effect	<i>d.f.</i>	<i>F</i> Ratio	<i>p</i>
Clutch	3	1.23	0.4893
Incubation temperature	2	3.57	0.0523
Clutch × incubation temperature	6	1.01	0.4283
Hormone treatment	3	1.91	0.1417
Clutch × hormone treatment	9	0.39	0.9381
Incubation temperature × hormone treatment	6	0.27	0.9483
Juvenile temperature	1	168.95	<0.0001
Incubation temperature × juvenile temperature	2	0.93	0.4010
Gonadal sex	1	1.04	0.3109
Previous growth rate (covariate)	1	8.63	0.0044
Residual	74		

Effect, source of variation; *d.f.*, degrees of freedom for effect; *F* Ratio, *F* statistic; *p*, probability of obtaining a value larger than the observed *F* Ratio. Details for calculating the *F* Ratio and the *d.f.* of the denominator for the mixed model analysis can be found in SAS (1989)

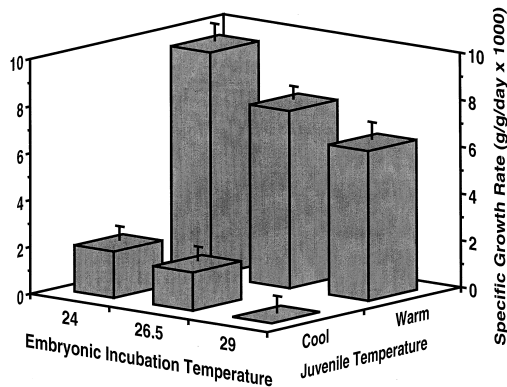


Fig. 1. Specific growth rate of hatchling snapping turtles (vertical axis) as a function of embryonic incubation temperature and juvenile holding temperature. Specific growth rates (g of mass gained/g of turtle/day) are least-squares means from the ANOVA described in the text and Table 1. Bars represent ± 1 standard error.

number of reasons. First of all, incubation temperature produced a comparable p -value in our earlier study of growth rates ($p = 0.0329$) even though the temperature effect on untransformed data (i.e., raw mass) was highly significant using a more powerful repeated measures analysis of variance ($p < 0.0001$). Likewise, smaller sample size in the current (20 individuals per temperature) versus the initial experiment (116 individuals per temperature) reduces statistical power. Thus, our new result is of biological significance despite the fact that the p -value is slightly above the standard critical value.

Although this result suggests that incubation temperature effects on growth are not mediated solely by

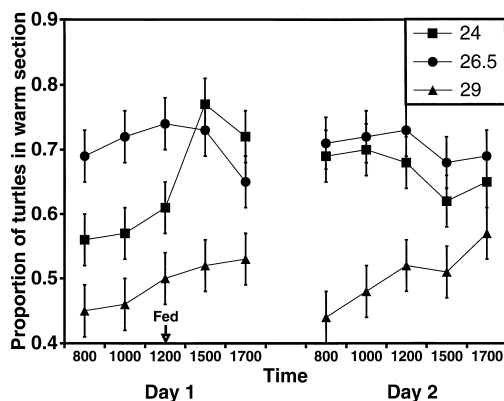


Fig. 2. Temperature choice as a function of embryonic incubation temperature. Temperature choice is presented as the average proportion of turtles from different incubation temperatures that were in the warm section of the gradient on consecutive feeding (day 1; $n = 13$) and non-feeding days (day 2; $n = 12$). Bars represent ± 1 standard error.

its effect on behavioral thermoregulation, a potential confounding factor is that turtles could thermoregulate prior to the current experiment. In an attempt to control for this effect, previous growth rate was used as a covariate. It is not surprising that this covariate was significant as repeated measurements of mass are often highly correlated. Further experiments should address whether prior thermal behavior contributes to this correlation. Nonetheless, other studies in which ambient temperature was held relatively constant support the hypothesis that incubation temperature influences growth potential independently of thermoregulation (Brooks et al., 1991; Bobyn and Brooks, 1994). For instance, thyroid hormones regulate growth in young turtles (Denver and Licht, 1991) and may mediate incubation temperature effects on metabolic rate in snapping turtles that are held at a constant temperature (O'Steen, 1994). However, our results also show that ambient temperature has a strong effect on growth.

At the most basic level, growth rate was seven-fold higher in turtles held at a constant high temperature (i.e., $\sim 28^\circ\text{C}$) than in turtles held at a constant low temperature (i.e., $\sim 19^\circ\text{C}$). Williamson et al. (1989) reported a similar result in snapping turtles collected from a natural nest with an unknown incubation history; turtles held at a constant temperature of 15°C did not grow whereas turtles raised at 25°C increased seven-fold in mass during 1 year. In other reptiles, the primary effect of higher body temperature is an increased rate and/or efficiency of digestion, potentially fueling higher growth rates (Kepenis and McManus, 1974; Harlow et al., 1976; Parmenter, 1981; Troyer, 1987; Zimmerman and Tracy, 1989; van Marken Lichtenbelt, 1992). Thus, embryonic temperature may affect growth by influencing both intrinsic growth potential and preferred body temperature. Indeed, access to a thermal gradient can increase growth rate in lizards (Sinervo and Adolph, 1994; Autumn and De Nardo, 1995) and fed turtles generally select higher body temperatures than do fasted individuals (Moll and Legler, 1971; Gatten, 1974; Hammond et al., 1988).

In our study, turtles from intermediate and low incubation temperatures were in the warm section of the thermal gradient more often than would be expected by chance, a pattern of temperature choice similar to that reported by O'Steen (1998). Although incubation temperature effects on growth and thermoregulation are correlated in both of these studies, the relationship between juvenile growth and thermal behavior is not as simple as this overall pattern would suggest. Our results also indicate that thermal selection by turtles from different incubation temperatures was dependent on time of day (i.e., turtles from 29°C moved into the warm section of the gradient during the course of the day), time after feeding (i.e., turtles from 24°C moved

into the warm section of the gradient after feeding), or was not associated with either of these variables (i.e., a constant proportion of turtles from 26.5°C were found in the warm section of the gradient). Thus, embryonic temperature effects on thermal behavior appear complex and must be fully characterized before any general relationships with growth can be established. To relate temperature choice to growth, growth must be measured at additional constant temperatures to determine the shape of the thermal performance curve for juvenile turtles from various incubation temperatures (Huey and Kingsolver, 1989).

The complex interaction among incubation temperature, nutritional status, time of day, and temperature choice described above may clarify data concerning thermoregulation in snapping turtles. Thermoregulatory behavior in this species has been studied by a number of investigators but with variable methods and levels of control. Obbard and Brooks (1979), for instance, reported that adult males bask more frequently than adult females in nature and found that cloacal temperature was strongly correlated with but significantly higher than mean ambient air temperature in 12 animals captured while basking. O'Steen's (1998) laboratory finding that incubation temperature, but not gonadal sex, influenced juvenile temperature choice suggests that this 'sex' effect may be due in part to the temperatures that these animals experienced as embryos. Although Schuett and Gatten (1980) determined more precisely the preferred temperature (~28°C) in two adult males and eight adult females in an artificial thermal gradient, they did not specifically test for any sex differences in this variable. Likewise, there was no control over sex or incubation temperature effects on preferred temperature in hatchlings collected from a natural nest (Williamson et al., 1989). Still, the mean selected temperature in these juveniles was similar to that found in adults.

In another study of juvenile snapping turtles, Knight et al. (1990) found that feeding did not increase thermophilic behavior. In that study, thermal choice was not allowed within the turtle's home cage, an important component known to alter appetite and thermoregulation (Regal, 1980). Interestingly, the turtles in the experiment by Knight et al. (1990) were incubated under a variable temperature regime: the range was 27–29°C. In our study, turtles from similar but constant incubation temperatures either showed no thermophilic response to feeding (i.e., turtles from 26.5°C) or circadian variation in temperature choice that was not related to nutritional status (i.e., turtles from 29°C). Such circadian cycles have been reported for females (i.e., turtles from warm incubation temperatures) in another species with TSD (Jarling et al., 1989). Brown and Brooks (1991) also reported that free-ranging adult snapping turtles did not display

thermophilic behavior in response to feeding, yet the incubation regime of these animals was unknown and cannot be inferred because their sex was not reported. In our study, only those turtles from the lower incubation temperature (i.e., 24°C) showed an association between nutritional status and temperature choice. This result may be more typical of reptiles since various turtles (Moll and Legler, 1971; Gatten, 1974; Hammond et al., 1988), lizards (Witten and Heatwole, 1978), crocodilians (Lang, 1979, 1981), and snakes (Kitchell, 1969; McGinnis and Moore, 1969; Van Meirop and Barnard, 1976; Regal, 1980) show thermophily after feeding.

Variation in juvenile temperature choice, which was induced by incubation temperature in the snapping turtle (O'Steen, 1998; this study), has implications for other TSD species as well. In the yellow slider turtle, gonadal sex interacts with nutritional status to affect basking behavior and body temperature in adults (Hammond et al., 1988). Satiated females prefer higher body temperatures than do satiated males. This sex effect, however, is less pronounced when turtles are fasted. Since sex is determined by incubation temperature in this species, it may be incubation temperature that affects thermoregulation rather than gonadal sex *per se*. Sex (or incubation temperature) and nutritional status also interact to influence behavioral thermoregulation in juvenile Siamese crocodiles, another reptile with TSD (Lang, 1987; Lang and Andrews, 1994). Notably, sex and nutritional status effects were reversed in this species such that males preferred higher body temperatures than females and feeding reduced, rather than increased, the sex difference. Although sex and incubation temperature effects are confounded in those studies, such effects may be due primarily to incubation temperature because sex had no effect on temperature choice (O'Steen, 1998) or growth (this study; Rhen and Lang, 1995) in turtles from the same incubation temperatures. Incubation temperature effects on thermoregulation and growth could influence size and age at maturity (Stearns and Koella, 1986). Interestingly, females are the larger sex in the yellow slider and males are the larger sex in the Siamese crocodile and the snapping turtle.

Overall, intermediate embryonic temperatures enhance juvenile growth in a variety of thermal holding conditions, under various feeding regimes, and in widely separated populations of snapping turtles (Brooks et al. 1991; McKnight and Gutzke, 1993; Bobyne and Brooks, 1994; Rhen and Lang, 1995; O'Steen, 1998; this study). If such incubation temperature effects persist beyond the young ages that have been examined so far, sexual size dimorphism in adult snapping turtles may be due in a large part to the matching of sexual phenotype to particular incubation conditions. Our results support this hypothesis because

intermediate embryonic temperatures produce males and because incubation temperature, but not gonadal sex, influences growth in young snapping turtles (Rhen and Lang, 1995; this study). Such temperature-induced correlations between growth and gonadal sex may be adaptive if large size and/or earlier maturity benefits males more than females (Charnov and Bull, 1977; Berry and Shine, 1980; Rhen and Lang, 1995). Our data are presumably relevant to snapping turtle thermal ecology, considering that the constant and thermal gradient temperatures we used cover the range of temperatures normally available to northern populations of the snapping turtle (Obbard and Brooks, 1979). In addition, the average selected temperature in free-ranging snapping turtles ($\sim 23\text{--}24^\circ\text{C}$; Brown and Brooks, 1991) was very similar to the average overall temperature in our thermal gradient ($\sim 24^\circ\text{C}$).

In summary, our results suggest that embryonic temperature directly affects growth potential in hatchling snapping turtles. Incubation temperature effects were still evident even when turtles were held at constant ambient temperatures. Incubation temperature may also affect growth through its effects on thermoregulatory behavior because turtles that select warmer body temperatures probably grow faster than turtles that select cooler body temperatures. Moreover, incubation temperature and nutritional status (or time of day) interact to produce diverse patterns of temperature choice that may reflect basic physiological differences among turtles from different incubation temperatures. Candidate factors that may mediate incubation temperature effects on growth and thermoregulation include pineal and thyroid hormones (Ralph et al., 1979; O'Steen, 1994; Geiser and Learmonth, 1994; Sinervo and Dunlap, 1995; Tosini and Menaker, 1996) and growth hormones (Gatford et al., 1998). Although our results have implications for the evolution of TSD and suggest that incubation temperature influences growth directly and through its effects on thermoregulation, there is a tremendous amount of work yet to be done on the thermal biology of reptiles with TSD.

Acknowledgements

We thank S. Swanson and M. Hermanson for assistance with data collection. We also thank A. Gluesenkamp, J. Sakata, and two anonymous reviewers for helpful comments on the paper. Support was provided by the National Science Foundation and Faculty Research Grants to J.W. Lang and a student research grant to T. Rhen from the Biology Department at the University of North Dakota. The Minnesota Department of Natural Resources provided a special permit (#6070) for egg collection.

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