UNIVERSITY OF OKLAHOMA

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HOW DEVELOPMENTAL ENVIRONMENT AFFECTS LIFE HISTORY IN BOX TURTLES

A Dissertation

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By

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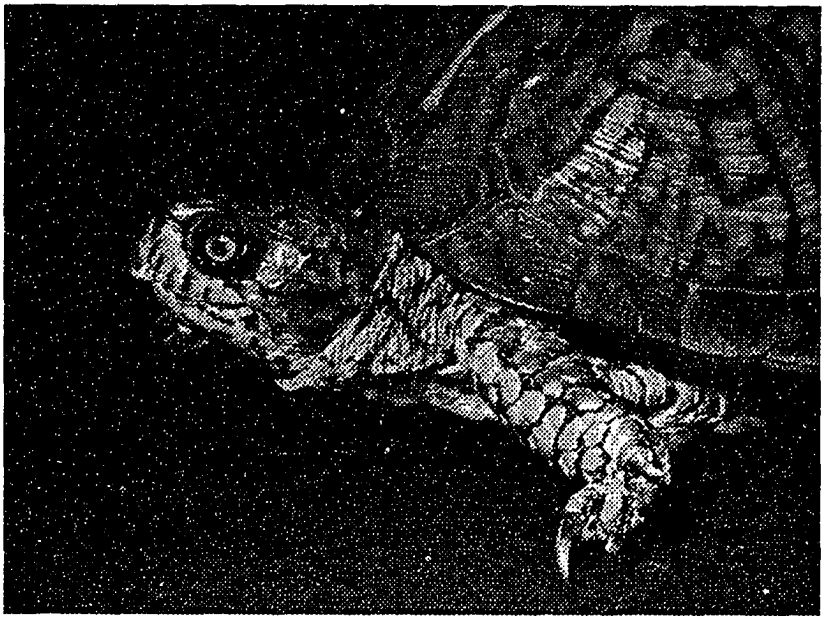


The turtle lives twixt plated decks
Which practically conceal its sex
I think it clever of the turtle
In such a fix to be so fertile
- Ogden Nash

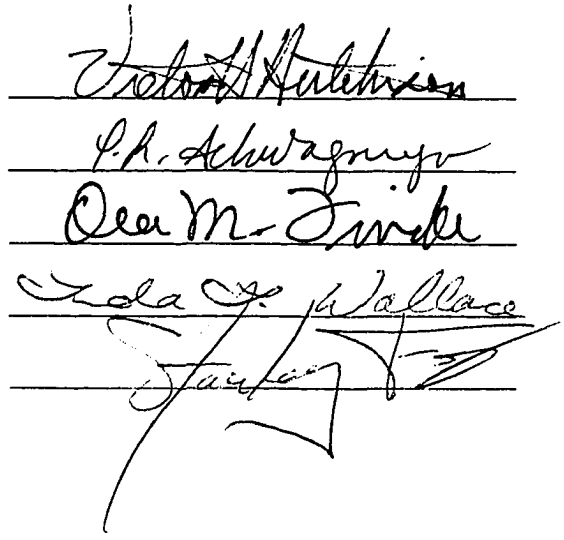
HOW DEVELOPMENTAL ENVIRONMENT AFFECTS

LIFE HISTORY IN BOX TURTLES

A Dissertation APPROVED FOR THE

DEPARTMENT OF ZOOLOGY

BY



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Finally I would like to thank Colleen for taking me to some wonderful field sites when my own field site consisted of Highway 9. She somehow felt that she was imposing on me by doing this; I did not discourage this view. She even took some of my teaching load in return for my "sacrifice". I would also like to thank my parents who encouraged my childhood interests in nature; I never grew out of them.

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Abstract

In box turtles (*Terrapene*), sex is determined by incubation temperature. An adaptive explanation is that, if (1) developmental environment influences growth and (2) larger size benefits one sex more then the other, then it is beneficial for developmental environment to determine sex because this allows a match of faster growth with the sex that benefits more from larger size. I tested some assumptions of this model in nature and in the laboratory, namely: (1) does larger size increase fecundity in females, the sex that is determined by higher incubation temperatures? (2) do females grow faster? (3) under controlled conditions, what are the effects of incubation temperature on growth and metabolic rate before hatching? and (4) what are the effects after hatching? I examined two species of box turtles *(Terrapene carolina triunguis and Terrapene ornata*) in Oklahoma.

There was no effect of maternal body size on egg size and number in either species. In addition, there was no difference in growth rates between the sexes in *T. ornata* and a significant difference in *T. carolina*. However, growth was fastest in *T. carolina* males, the sex that results from *lower* incubation temperatures. In both species, females are larger than males because they delay maturity rather than grow faster.

In the laboratory, I controlled developmental temperature and measured size at hatching and metabolic rate of eggs. Eggs were incubated at 25 C and 30 C to produce males and females, respectively. Hatchlings from lower incubation

temperature (males) were slightly larger and consumed more oxygen throughout development. The hatchlings were then raised under common conditions. After two years, growth differed between species but not sexes. Metabolic rate at 20 C, 25 C, and 30 C differed significantly between the sexes.

In conclusion, a simple relationship between incubation temperature and body size does not exist in nature or in the laboratory. Reproductive output is little affected by body size and adult body size is determined by timing of maturation rather than growth rate. Under controlled conditions, incubation temperature does not affect subsequent hatchling growth, nevertheless, it does affect size at hatching, energetic costs, and metabolic rate.

Chapter 1 . Egg size, clutch size, and body size in two species of box turtles (*Terrapene*)

Abstract

An increase in the value of one desirable trait may drive a decline in another, a trade-off. Among life history traits, one such trade-off is between size and number of offspring. I examined how maternal body size affected egg size and number in two species of box turtles (*Terrapene carolina triunguis* and *Terrapene ornata*) in Oklahoma. There was no significant correlation between egg size and number in either species, even after the effect of maternal body size was removed. Number of years in captivity had a negative effect on egg size in *T. carolina* and clutch size in *T. ornata*. Maternal condition was positively correlated with egg mass but not clutch size in *T. ornata*. There was a trade-off between egg size and number in *T. ornata* that only became apparent after the effect of maternal condition was removed. Overall, the smaller species, *T. ornata*, laid a larger clutch mass on average because eggs were larger rather than more eggs per clutch. These analyses illustrate some difficulties of determining trade-offs on the physiological, population, and species levels.

Introduction

"A trade-off occurs when increase in one thing implies a decrease in something else" (Stearns 1992). These trade-offs can occur at several different levels. On an

individual level, different processes (i. e., growth or reproduction) may compete for energy within an animal or plant (physiological trade-offs, Stearns 1992). These compromises do not necessarily have a genetic basis. On a population level, two traits, each of which on its own enhances fitness, may negatively covary (microevolutionary trade-offs, Stearns 1992). If this covariance is genetic, selecting for one trait may necessarily cause the other to decrease. If these traits become fixed within species, comparisons between species may reveal the history of selection on these trade-offs (macroevolutionary trade-offs, Stearns 1992). Each of these levels is nested within the next higher level: population level trade-offs include physiological trade-offs, and species level trade-offs include microevolutionary trade-offs. Therefore, any comparison at any level must include environmental and genetic effects and their interaction.

The covariance between two traits may be difficult to detect because any two traits belong to a suite of correlated traits (van Noordwijk and de Jong 1986, Roff 1992). Van Noordwijk and de Jong (1986), used the following example. Within an individual, growth and reproduction compete for resources. If one could contrast energy devoted to growth and energy devoted to reproduction in a random sample of individuals, the correlation between these traits may reveal this trade-off. However, animals that acquire more resources may both expend more on growth and expend more on reproduction and animals that acquire fewer resources may expend less on both. The covariance of growth and reproduction could be positive. A negative

correlation may be apparent only if variation in acquisition of resources is considered or if animals are measured under stress.

One of the major trade-offs is between offspring size and number. If larger offspring survive better, selection for production of larger offspring is offset by the disadvantage of producing fewer offspring. The compromise is between fitness of each offspring, which is proportional to energy invested in each offspring, and fitness of the mother, which is a product of offspring fitness and number of offspring produced (Smith and Fretwell 1974). The optimal trade-off is the point at which the maternal fitness is tangential to the average fitness of offspring. However, assumptions of the model include no other trade-offs for the mother, for example, current reproduction vs. future reproduction. Correlation of offspring size and number entails the aforementioned problem of a third variable that confounds the relationship between egg number and size, in this case total investment in offspring. Where total investment varies among individuals (as a function of time, body size, age, or condition), the trade-off between size and number may be obscured.

A second issue is the influence of body size on reproductive output in turtles with environmental sex determination. One explanation for the evolution of environmental sex determination hinges on differences in size advantages between the sexes (Charnov 1982, Janzen and Paukstis 1991). The explanation is that if developmental environment enhances size and size advantages are asymmetric between sexes, then it is advantageous for environment also to determine sex; this allows a

match of sex to size (Charnov and Bull 1977). Both species are sexually dimorphic and have environmental sex determination. By examining the slope of the relationship between reproductive output and body size, I try to describe size advantage to the larger sex (females) in two ecologically-distinct species of box turtles (*Terrapene carolina triunguis* and *Terrapene ornata*). I also examine some trade-offs between egg number and size within the context of maternal body size and condition.

Materials and Methods

Subjects.

I examined two species of terrestrial turtles in the genus *Terrapene*, family Emydidae: the three-toed box turtle, *T. carolina triunguis*, and the ornate box turtle, *T. ornata*. *T. carolina* is primarily a woodland species and *T. ornata* is primarily a prairie species (Ernst and Barbour 1972, pers. obs.) but their geographical ranges overlap in central Oklahoma where I collected specimens. I collected gravid females on the roads in the spring; some were held in outdoor pens at the Animal Behavior Facility, University of Oklahoma. I found gravid females only at the beginning of the summer. I collected eggs as part of an inquiry into incubation effects on growth and development (St. Clair, unpubl.). I induced oviposition by injection of oxytocin (100 I.U. per ml, 0.01 cc per 100 g body mass) and, at laying, measured eggs and post-partum mass of mothers. Egg mass at laying was assumed to be a reasonable estimate of parental investment because all eggs were at the same stage of development (turtle

eggs are suspended in the gastrula stage until laying, Ewert 1985). Here I use "clutch size" to refer to number of eggs in a clutch or fecundity, not clutch mass; egg size refers to individual egg mass at laying. I compared two species within the same genus to reduce confounding effects of phylogeny; any difference between them must have arisen since their last common ancestor, thus limiting the number of confounding covariates (Harvey and Pagel 1991).

Effect of size.

I tested the effect of body size on reproductive output (clutch mass, egg mass, egg number) to assess the advantage of larger size to the females. Because clutch mass is the product of clutch size (n) and average egg mass (m_E), I used the following models to test if maternal body mass was proportional to clutch mass, i. e.,

$$n \times m_E \propto m_B \qquad (1)$$

I converted (1) to a linear equation using natural logarithms:

$$\ln n + \ln m_E \propto \ln m_B \qquad (2)$$

To derive the following multiple linear regression model:

$$\ln m_E = a + b_1 \ln m_B - b_2 \ln n \qquad (3)$$

where m_E is egg mass (average per clutch), a is a constant, m_B is maternal body mass, and n is number of eggs. Clutch size (n), is on right of equation because number of eggs to body mass correlation was low and therefore the likelihood of there being a

linear relationship among independent variables (multicollinearity) was reduced. Partial regression coefficients (b) of the predicted sign (positive or negative) indicate: egg mass decreases with increased clutch size (number of eggs) when separated from the influence of body size, and egg mass increases with increased body mass when separated from the influence of clutch size (Lewis-Beck 1980). I rearranged (3) so that ln n + ln m_E (= ln of clutch mass) is on the same side of the model,

$$\ln m_B = a + b_1 \ln m_E + b_2 \ln n \qquad (4)$$

A positive correlation coefficient (r) therefore indicates increase of ln(clutch mass) with ln(body mass).

Effect of condition and captivity

Because some animals had spent up to three years in pens (14 of 27 *T. carolina* and 3 of 14 *T. ornata*) and some were freshly caught, I wished to examine the influence of egg size on number while removing the influence of time in captivity and condition of the mother. I used a multiple linear regression:

$$n = a + b_1 t + b_2 m_E + b_3 m_B + b_4 c \qquad (5)$$

where n is number of eggs, a is a constant, t is time (yr.) in captivity, m_E is egg mass (average per clutch), m_B is body mass of mother, and c is condition of mother. The model for egg mass as the dependent variable was similar, but with m_E and n interchanged. I used residuals from regression of ln(body mass) on ln(length) as an

estimate of condition, i. e., animals that are heavier than average for their length will have positive residuals.

Comparisons between species.

Individual egg mass, number of eggs per clutch, and clutch mass were compared between species using ANOVA. Because eggs were grouped within clutches, clutch (nested within species) was an additional main effect when testing difference of egg mass between species. Clutch effect is therefore a mixture of maternal provisioning, and maternal and paternal genetic effects.

Statistics.

I used general linear models (Wilkinson 1990) to compare main and covariate effects. The assumption of homogeneity of slopes in analysis of covariance was tested by examining the interaction between the covariate and main effects. Where possible, the independent variable in least-squares regression was the one measured with the least error (e. g., number of eggs instead of egg mass). All summary statistics are means and standard errors unless otherwise noted.

Results

Effect of size

Regression coefficients were in the predicted direction but none were significant (Table 1, Fig. 1, Fig. 2). The correlation coefficient between clutch mass

and body mass was also nonsignificant (Table 1). However, powers of the tests were below the desired level of 0.80.

Effect of condition and captivity

Body mass of the mother did not significantly influence either egg mass or egg number. Years spent in captivity negatively affected egg mass in *T. carolina* and number of eggs per clutch in *T. ornata* (Table 2). In *T. ornata*, better condition of mother also positively influenced number of eggs per clutch. When the influence of condition of mother or time in captivity was removed, there was a negative relationship between egg size and number in *T. ornata* (Table 2), an effect that could not be distinguished when considering body mass alone (see above).

Three individuals produced a clutch when captured and another after being held in captivity. The second reproductive bouts were not included in the above analyses but I examined them for individual trade-offs between egg size and number. In all cases, length of mother remained the same but mass changed between laying dates; one female lost 48 g between producing clutches in 1991 and 1993, the other two gained mass (36 g: 1992-1994, 21 g: 1993-1994). The individual that was in poorer condition after two years laid four eggs each time but produced smaller eggs the second time (8.25 g vs. 9.11 g, ± 0.25 SE). Both individuals that gained in mass, increased their clutch size at the expense of egg size (clutch size: 5 vs. 4 and 4 vs. 3, egg mass: 10.2 ± 0.23 vs. 10.95 ± 0.25 and 9.11 ± 0.25 vs. 9.99 ± 0.29); however,

clutch mass was larger the second time. This contrasts with *T. ornata*, measured at the population level, in which mothers in better condition laid larger eggs when the influence of clutch size was held constant.

Species comparisons

There was a significant difference in egg mass among clutches ($F_{40,119} = 22.35$, $p < 0.001$). Although considerably smaller, *T. ornata* produced significantly larger clutch mass on average (42.50 ± 2.18 vs. 35.43 ± 1.57, $p < 0.001$, Fig. 3). This difference was due mostly to *T. ornata* producing larger eggs (10.51 ± 0.05 vs. 9.38 ± 0.07, $F_{1,119} = 203.31$, $p < 0.01$, Fig. 2). Median number of eggs was 4 for both species (Kruskal-Wallis test, $H = 2.25$, $p = 0.134$, Fig. 1) although *T. ornata* produced slightly more eggs on average.

In both species, there was a significant difference among clutches in both egg length ($F_{40,122} = 15.60$, $p < 0.001$) and egg width ($F_{40,122} = 6.074$, $p < 0.001$). Egg width was slightly larger in *T. ornata* than in *T. carolina* (21.776 ± 0.079 vs. 21.410 ± 0.061, $F_{1,122} = 19.81$, $p < 0.001$, $n = 59$, 105 respectively, Fig. 4). Egg length was also larger (37.16 ± 0.175 vs. 35.01 ± 0.133, $F_{1,122} = 105.14$, $p < 0.001$, Fig. 4).

Discussion

Maternal body size had no significant effect on egg size, clutch size (number of eggs per clutch), or clutch mass. When the effect of body size was statistically

removed, average egg size did not decrease with increased clutch size. However, in *T. ornata*, when condition of the mother was introduced as an independent variable, average egg size decreased with increased clutch size. Better condition of the mother was associated with larger eggs but not more eggs per clutch. In both species, clutch size decreased on average with increased years in captivity. Larger clutch size was associated with smaller average egg size when the influence of number of years in captivity was statistically controlled, but only in *T. ornata*. In comparing the two species, *T. ornata*, although smaller, produced a larger clutch mass per reproductive bout. Clutch mass was larger because the eggs were larger, rather than because there were more eggs per clutch. The eggs were larger principally because they were longer. However, although turtles of both species lay eggs at most once per year, I have no idea if clutches are produced each year in this population. In Kansas, Legler (1960) reported that *T. ornata* females produced a clutch annually and in some cases (3 of 11), twice a year.

In *T. ornata* from Kansas, Legler (1960) reported that increase in clutch size with increasing maternal body length was not pronounced. Although this was not tested statistically, the relationship seems as weak as in this sample (cf. Fig. 6, Legler 1960). Egg dimensions were remarkably similar to the Oklahoma sample (means ± SD, mass: 10.09g ± 1.31, length: 36.06 ± 2.77, and width: 21.72 ± 1.04). He remarked that the smallest clutch (2 eggs) had the largest eggs and the largest clutch (8 eggs) had the smallest eggs.

Both *T. carolina* and *T. ornata* are sexually dimorphic species; females are larger on average than males because they delay maturity rather than grow faster (St. Clair, unpubl.). Because delaying maturation lowers the probability of surviving to reproduce, females that mature older or larger might be expected to produce either better quality or more offspring (Bell 1980, Stearns 1992). In this study, egg mass and clutch size varied widely over the range of body sizes and therefore I could detect no significant effect of maternal body size per se on reproductive potential. However, there may be a difference between rate at which fecundity increases with size or age prior to maturation and rate of increase thereafter (see Linear Fecundity Model, Stearns 1992). Most females in my sample were probably well past the minimum age of first reproduction. One way to estimate the advantage of delaying maturation would be to examine a sample of females at first reproduction for a positive relationship between size and fecundity. Alternatively, examining fecundity in females in which maturation was experimentally delayed or advanced would more firmly establish a link between delayed maturation and increased fecundity (*sensu* Reznick 1985).

On the simplest level, number of offspring may affect offspring size because volume within the mother is limited. The trade-off is ultimately between the reproductive advantage of producing larger offspring and the disadvantage of producing one fewer offspring (e. g., Smith and Fretwell 1974, Brockelman 1975, Lloyd 1987). However, as with any trade-off, this may be measured at the individual,

population, or above the species level (physiological, microevolutionary, and macroevolutionary trade-offs, Stearns 1992). Each level subsumes the next, for example, population level trade-offs include the individual level, and species level trade-offs reflect a history of individual and population level trade-offs. At the lowest level, physiological trade-offs involve individual compromises in allocation of energy. An example from this study is the three *T. carolina* that changed in condition between two reproductive bouts. The individual that lost mass produced the same number but smaller eggs. The two individuals that gained mass produced more but smaller eggs; total clutch mass increased. One explanation for smaller eggs with better condition of mother is that increase in clutch size is incremental. In other words, clutch mass may increase by increased egg size until a threshold egg size is reached and then an extra egg is produced. Although these observations may not be representative of physiological trade-offs in general, they illustrate the perils of examining the trade-off between only two variables, regardless of others.

At the population level, there was no evidence of a trade-off between egg size and number within each species. However, if another variable (e. g., body size) is correlated with either egg size or number and there is a large variation in body size, this trade-off could easily be obscured (van Noordwijk and Jong 1986, Roff 1992). For example, if clutch size is positiveiy related to maternal body size, but larger individuals also produce larger eggs, then a sample of the population may show a positive correlation between egg size and number (Roff 1992). I therefore controlled

for the effect of body size using (1) and there was still no significant decrease in egg size with number of eggs in a clutch. However, instead of body size, two other variables were correlated with egg size and number. After the influences of either body condition or years in captivity were statistically removed, the expected trade-off was apparent, at least in *T. ornata*, the smaller species.

These two species also illustrate how the description of a trade-off in egg size and number by phylogenetic comparisons is risky, even when taking body size into account. *T. ornata* is much smaller but produces at least as many eggs as *T. carolina* and the eggs are larger. Including these two species in an analysis, especially using residuals from body size regressions, would tend to flatten or make positive any correlation between egg size and number. *T. ornata* has both large egg size and number compared with *T. carolina* and this would tend to reduce negative correlations. The independent contrast (Felsenstein 1985) would also be large because the two species are within the same genus. However, Elgar and Heaphy (1989) found a trade-off between egg size and number in turtles.

The above examples illustrate some of the pitfalls of interpreting phenotypic correlations, e. g., incomplete knowledge of confounding variables. Major problems remain such as interpreting causation from correlation and absence of knowledge of genetic control of life history traits and their covariance (Reznick 1985).

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Tables

Table 1. Egg size, number of eggs per clutch, and body size in *T. carolina* and *T. ornata*. P-values are in parentheses; r-values (rather that R^2) are reported to indicate the sign of the relationship between maternal body mass and clutch mass (see text).

Species	N	constant	body mass	fecundity	r	Power
T. carolina	24	0.55	0.28	-0.02	0.30	0.37
		(0.73)	(0.20)	(0.15)	(0.37)	
T. ornata	14	0.52	0.37	-0.19	0.53	0.50
		(0.64)	(0.09)	(0.21)	(0.17)	

Table 2. Partial regression coefficients for multiple linear models. Dependent variables are n, number of eggs, or m_E, average mass of eggs (per clutch); a is a constant, t is years in captivity, and c is condition. P-values are in parentheses and significant coefficients are in bold type.

Species	Dep. var.	a	t	n	m_E	m_B	c	R^2
T. carolina	n	-0.72	0.08	---	0.33	0.20	-0.50	0.10
		(0.77)	(0.21)		(0.48)	(0.58)	(0.32)	(0.72)
n = 24	m_E	1.843	**- 0.09**	0.08	---	0.06	0.10	**0.41**
		(0.12)	(0.00)	(0.48)		(0.74)	(0.71)	(0.01)
T. ornata	n	**7.02**	**- 0.77**	---	**-0.26**	0.20	0.22	**0.44**
		(0.00)	(0.06)		(0.047)	(0.54)	(0.50)	(0.04)
n = 14	m_E	**14.16**	-0.13	**- 0.94**	---	0.13	**8.92**	**0.28**
		(0.00)	(0.67)	(0.04)		(0.70)	(0.03)	(0.01)

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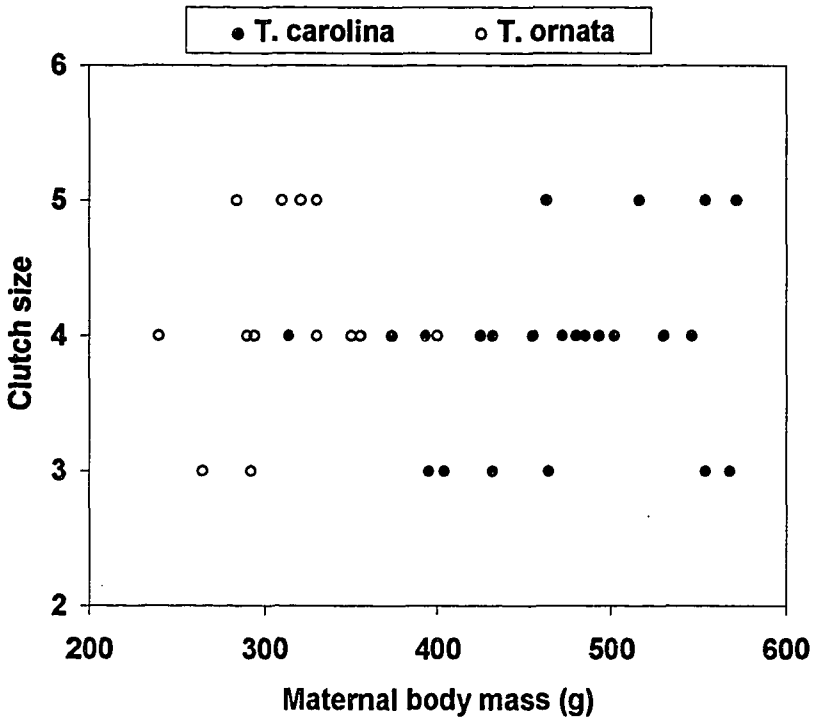

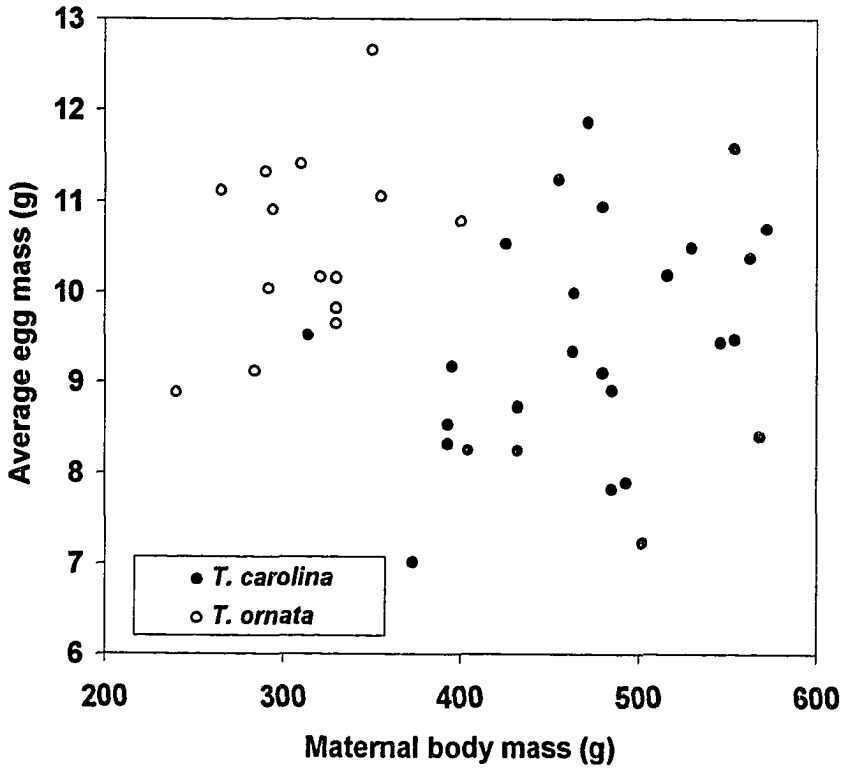

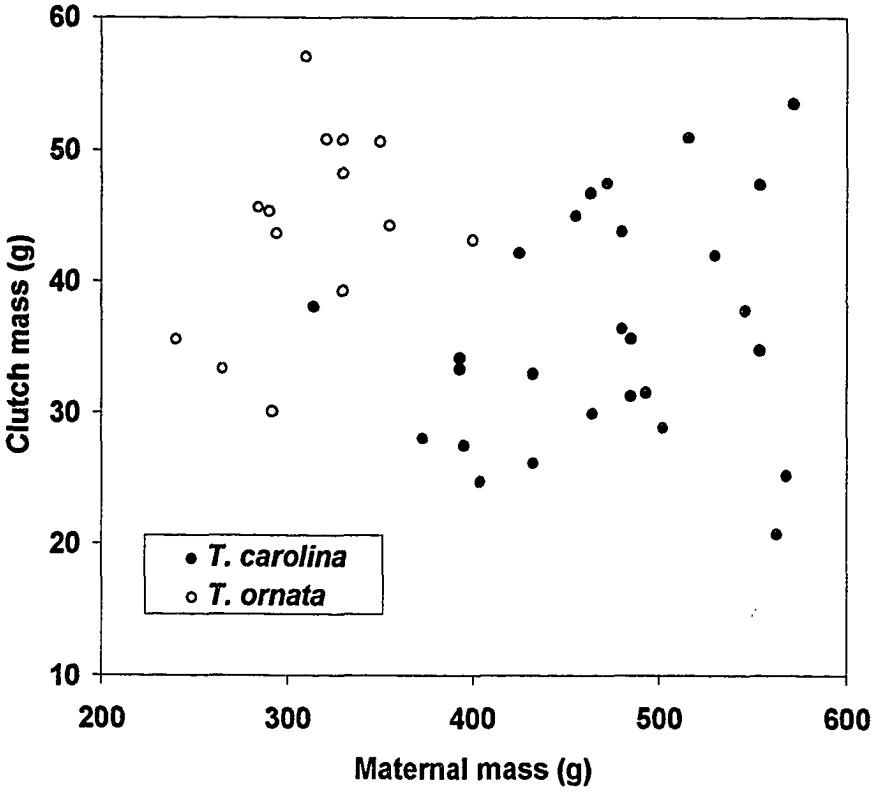

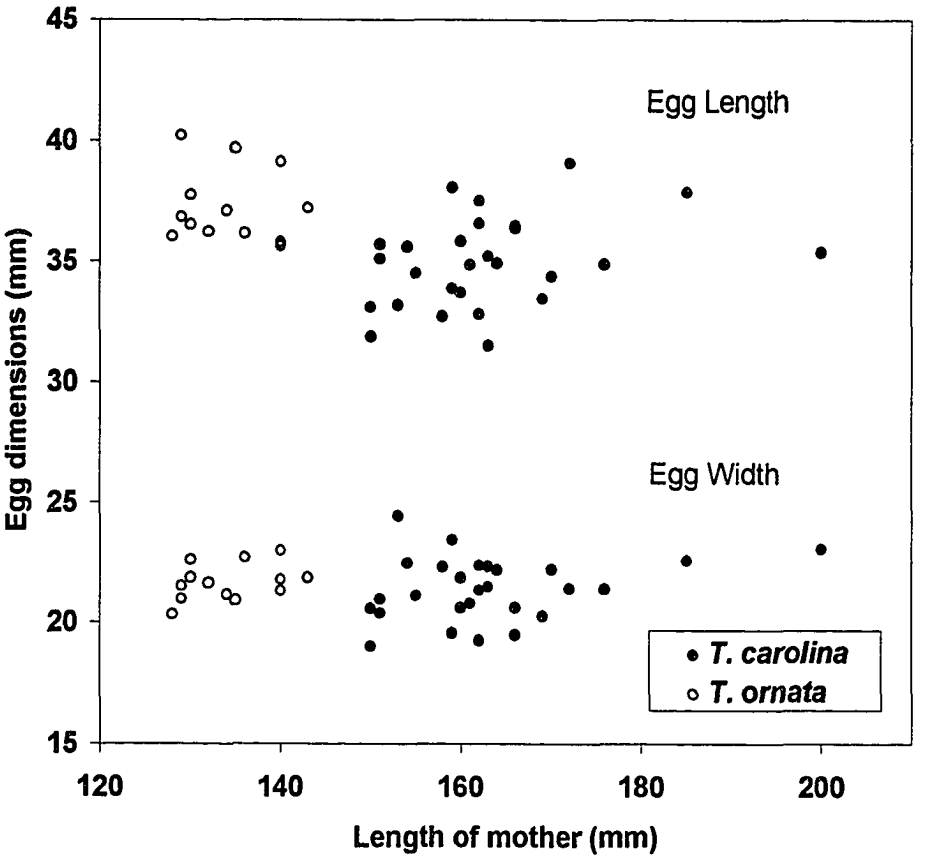

Chapter 2 . Sexual differences in growth in two species of box turtle (*Terrapene carolina* and *T. ornata*)

Abstract

An adaptive explanation for environmental sex determination is that, if the developmental environment imposes asymmetries in growth, then it is beneficial to allow a match of faster growth with sex when larger size benefits one sex more then the other. However, individuals may be larger because they delayed maturity or grew faster and, therefore, sexual size dimorphism can be understood only within the context of both growth and maturation. In addition, sexual size dimorphism at maturity in those animals that continue to grow after maturity could change as growth competes for energy with reproduction and maintenance. I compared growth using annuli on carapace scales in two species of box turtles (*Terrapene carolina* and *T. ornata*) that have similar patterns of environmental sex determination but reportedly have different patterns of sexual size dimorphism. However, in the populations I studied, adult females were, on average, larger than adult males in both species. This was because males matured earlier and therefore at smaller sizes than females. In *T. carolina*, males grew faster than females as juveniles but females had the larger asymptotic size. In *T. ornata*, males and females grew at similar rates and had similar asymptotic sizes. Sexual size dimorphism decreased after maturity because males grew more as adults. There was therefore no consistent pattern of faster growth for females that may be ascribed to the developmental temperature.

Introduction

Benefits of large size may differ between the sexes. For example, females may benefit more from large size than males because they must carry young or eggs. Alternatively, males may benefit more from large size because of contests with other males for mates and territory or because of a female preference for large males (Darwin 1871). A third possibility is that the sexes differ in size when they occupy different ecological niches (Darwin 1871, Slatkin 1984, Shine 1989). An individual is larger than another because it started out larger, grew faster, or grew for a longer time (delayed maturity). Larger initial (propagule) size means the parent has invested more per individual offspring at the expense of number of offspring (Wilbur 1977, Lloyd 1988, Winkler and Wallin 1987). Faster growth means that energy must be diverted from maintenance or reproduction and thus survival or fecundity is compromised (Kozlowski 1992); delayed maturity carries the penalty of a lowered chance of surviving to reproduce (Bell 1980). These trade-offs may be sex-specific and may be reflected in sexual size differences.

The basis for sexual size dimorphism may differ depending on growth patterns. Lifetime patterns of growth fall into two broad categories: growth ceases before or at sexual maturity (determinant, e. g., birds, insects, some mammals) or growth continues after sexual maturity (indeterminate, e. g., reptiles, amphibians, crustaceans, most fish). In the first case, average adult size reflects selection on juvenile growth and maturation (Stamps 1983, Shine 1990). Increased juvenile growth often means earlier maturation because the marginal size for reproduction is reached earlier (e. g., Roff 1992, Charlesworth 1994). After maturity, size is fixed and energetic trade-offs are among

maintenance, storage, and reproduction. However, in animals that continue to grow after sexual maturity, average size also reflects trade-offs between adult growth and current reproduction (Stamps 1983, Shine 1990). Therefore, changes in sexual size dimorphism after maturity offer a clue to the significance of size differences between the sexes.

The average of any sample of sizes among mature individuals will depend on age distribution and hence on survivorship (Dunham and Gibbons 1990, Stamps 1993). For example, if smaller males suffer higher mortality than larger males after maturity and there is no difference in females, then males will be larger than females on average (e. g., Sinervo et al. 1992), even if male and female growth and age at maturity are the same. Second, even if survivorship does not differ among size classes within each sex, sex differences in survivorship (i. e., costs of reproduction, Shine 1980) can create sex differences in average size. This is because there will be fewer large, old individuals in the sex with the lower annual survivorship. This can be illustrated by substituting length for age in a simple exponential model of survivorship (Appendix). The shape of the distribution of sizes depends on the ratio between survivorship, M, and growth rate, K. Mean sizes in the population become quite different even when growth rate and asymptotic length are the same (Fig. 1).

Finally, adult size may be determined early in life if developmental environment determines juvenile growth and maturation. If sexual differences in adult size are adaptive (i. e., reflect asymmetries in fitness advantages of size between the sexes) and developmental environment affects adult size, it may be advantageous for developmental environment to also determine sex (Charnov and Bull 1977). Patterns of sexual size

dimorphism match developmental environment and sex determination in turtles and crocodiles; the larger sex generally comes from hotter nests (Head et al. 1987). The comparison between the two groups is illuminating because sexual size dimorphism differs; males are larger in crocodiles and females are larger in turtles. An adaptive explanation for sexual size dimorphism is that male crocodiles compete with each other for territories and mates and female turtles increase reproductive output with size (Head et al. 1987, Berry and Shine 1980). If patterns of growth differ between the sexes, this is a possible link between development and sexual size dimorphism. However, there are several species of turtles that have reversed sexual size dimorphism in which males are larger than females but the same pattern of environmental sex determination in which females are larger; this belies a simple relationship between growth and developmental temperature (Janzen and Paukstis 1991). Comparisons of growth patterns between species with different patterns of sexual size dimorphism but similar patterns of environmental sex determination may be informative; if reversals of sexual size dimorphism are due to delayed maturity and not faster growth in the larger sex, a link between developmental environment, growth, and sexual size dimorphism may still be plausible.

Here, I examined growth and size in two species of box turtles (*Terrapene carolina triunguis* and *T. ornata*) that were reported to display different patterns of sexual size dimorphism (Janzen and Paukstis 1991: *T. ornata* equal sizes, *T. carolina*, males larger than females, but see below) and yet have the same pattern of environmental sex determination. I examined: (1) differences in growth rate and age at maturity between the

sexes to see if growth patterns were sufficient to explain sexual size dimorphism without reference to survivorship, (2) changes in sexual size dimorphism after maturity as an indirect way to compare differences in costs of reproduction between the sexes, and (3) differences in growth to see if a link between developmental environment, growth, and sexual size dimorphism is plausible. The basis for this inquiry is that sexual size dimorphism in animals that continue to grow after maturity can only be understood within the framework of patterns of growth and maturation (Stamps 1993).

Materials and Methods

Subjects

I examined two species of terrestrial turtles in the genus Terrapene, family Emydidae: the three-toed box turtle, T. carolina triunguis, and the ornate box turtle, T. ornata. T. carolina is primarily found in woodlands and T. ornata is primarily found in grasslands (Ernst and Barbour 1972, pers. obs.) but their geographical ranges overlap in Oklahoma where the specimens were collected. Turtles were mostly collected crossing roads in the spring. I compared two species within the same genus because any difference between them must have arisen since their last common ancestor thus limiting the number of confounding covariates (Harvey and Pagel 1991). These species are particularly well suited for growth studies because they retain a record of growth on their shells. It is therefore possible to calculate size at previous ages using standard fisheries techniques for determining growth from annuli on scales (Schreck and Moyle 1990).

Size at sexual maturity

To determine size and age at sexual maturity, I constructed size and age frequency histograms. I identified males by secondary sexual characteristics (reddish iris, concave plastron, short, curved hind claws, and reddish head in T. c. triunguis or greenish head in T. ornata) and defined onset of sexual maturity for males as the minimum size or age at which individuals possessed these features. Because adult females are externally indistinguishable from juveniles except by size (the variable I was trying to measure), I defined onset of sexual maturity in females by the smallest/youngest gravid female. I measured the changes in sexual size dimorphism after maturity by comparing dimorphism at maturity and average dimorphism after maturity, calculated from average size in the population.

Annuli as valid indicators of age

I first tested two assumptions of using lines on scales (circuli) to back-calculate size at previous ages i. e., are circuli deposited annually (are they annuli, do they indicate age) and is scale radius a good predictor of size (Schreck and Moyle 1990)? To test if scale radius was a good predictor of size, I regressed length of shell on scale radius. Length (L) was measured along the curve of the carapace with a tape measure; scale radius (R_s) was measured from the focus (start of growth) to the distal edge of the largest scale on the carapace (right second pleural). I looked for differences between species and sexes (male, female, and juvenile) using ANCOVA. Species differed ($p = 0.001$) and were considered separately; sexes did not and were pooled. In T. carolina scale radius accounted for 93% of the variance in carapace length ($L = 42.88 + 2.80R_s$, $p < 0.001$, n =

227) and in T. carolina, 95% of the variance in carapace length ($L = 22.09 + 3.07R_s$, $p < 0.001$, $n = 127$). Because the correlation between scale radius and length was large, I chose not to back-calculate length at previous ages. Analyses were therefore performed on annulus radius not estimated carapace length. However, in the figures, I transformed annulus radius to carapace length using the above regressions to illustrate asymptotic length.

To test if major circuli were valid indicators of age, I examined animals that were held in pens (200 T. carolina, 114 T. ornata) to test if circuli on scales were deposited annually (= annuli). Circuli were deposited annually but only in growing animals. Among older animals, circuli were not deposited or were too close together to measure; the maximum number of annuli that I could count was 14. Extra circuli were shallower, wore off with age, and could usually be distinguished from annuli. Because animals in pens were held under close to natural conditions (e. g., they hibernated), I assumed that wild caught individuals also followed this pattern.

Growth models

Change in length was fit to the von Bertalanffy model (von Bertalanffy 1968) using non-linear regression:

$$L = A(1 - e^{-Kt}) \qquad (1)$$

where A is asymptotic length, K is the rate of approach to asymptotic length and t is age. Originally, the von Bertalanffy model was derived from physiological principles describing catabolism and anabolism of tissue (von Bertalanffy 1968, Reiss 1989). Raising

this curve by some exponent (usually close to 3) converts growth by length to growth by mass and generates the family of sigmoidal growth curves (e. g., logistic, Gompertz, Andrews 1982). Because length is usually measured after hatch or birth, the equation is often modified by incorporating a third parameter (t_0 - the hypothetical time at length zero) that moves the curve along the time axis:

$$L = A(1 - e^{-K(t-t_0)}) \qquad (2)$$

Using a third parameter allows for comparisons with animals in which growth measurements start at a size (size at hatch) that is large relative to adult size (e. g. reptiles, Charnov 1993). This parameter can be estimated but I used incubation times from the laboratory (St. Clair, in prep.) at 25 C (75 days, male-producing temperatures) and 30 C (50 days, female-producing temperatures). Here, t_0 is negative because hatchlings were considered to be age zero. At any rate, any error due to t_0 is small because t_0 is insignificant compared to the ages of the turtles.

Nonlinear regression was only useful to estimate parameters of the model because assumptions of regression analysis are violated when individuals are measured repeatedly and when variance in length (the dependent variable) increased with time (the independent variable); this is a common problem with growth analyses. F-tests of significant differences of parameters are therefore suspect although estimates of the parameters are accurate (Horton 1978). To confirm predictions of the growth model, I used repeated measures ANOVA and MUDIFT (multivariate distribution-free comparison of growth curves, a non-parametric technique for comparing median size at each age (Dallal et al. 1989).

Statistics

General linear models (Wilkinson 1990) were used to compare main and covariate effects. The assumption of homogeneity of slopes in analysis of covariance was tested by examining the interaction between the covariate and main effects. All summary statistics are means and standard errors unless otherwise noted.

Results

Sexual size dimorphism

In both species, males matured at smaller sizes and younger ages than females (Fig. 2, Table 1). On average, adult females were significantly larger than adult males in both species (*T. carolina*, $F_{1,165} = 65.90$, $p < 0.001$; *T. ornata*, $F_{1,106} = 48.58$, $p < 0.001$) and sexual size dimorphism decreased after maturity (Fig. 3). Size differences between the species increased.

Growth

The parameters of the von Bertalanffy model indicated that, in *T. carolina*, males grew faster and had a smaller asymptotic length but there was little difference between male and female growth rates or asymptotic length in *T. ornata* (Table 2, Fig. 4). These results agreed with the MUDIFT analysis; median size was significantly larger in *T. carolina* males up to 10 years old ($\chi = 7.99$, $df = 2$, $p = 0.018$, $n = 32$ females, 36 males) after which size differences were not detectable; there was no significant difference in *T. ornata* ($\chi = 0.47$, $df = 2$, $p = 0.791$, $n = 37$ females, 13 males). Repeated measures

ANOVA gave similar results; mean size was significantly larger in *T. carolina* males up to 6 years old ($F_{1,57} = 6.29$, $p = 0.015$, n = 28 females, 31 males) after which differences were not detectable and there was no significant difference in *T. ornata* ($F_{1,44} = 0.00$, $p = 0.986$, n = 35 females, 11 males).

Discussion

These two species illustrate two patterns of growth; in one, growth trajectories are similar between the sexes (*T. ornata*) and in the other, both parameters differ between the sexes but with larger asymptotic length (A) associated with lower k (Fig. 5, bottom-right, *T. carolina*). In the first case, *T. ornata*, sexual size dimorphism can be explained by differences in age at maturity. Average size for males is smaller because a sample of males will include younger individuals. In contrast to males, *T. ornata* females grow little after maturity (Fig. 4), perhaps because a larger proportion of energy is allocated to reproduction. In the second case, sexual size dimorphism can again be explained by age at maturity but the larger sex, female, approaches asymptotic size at a slower rate. Rapid juvenile growth is often associated with early reproduction and smaller asymptotic size (Gadgil and Bossert 1970, Charlesworth 1994, Charnov 1993; examples: Stearns 1983, Reznick and Bryga 1987, Lovich et al. 1990, Clutton-Brock et al. 1982). In both cases, patterns of growth and maturation seem adequate to explain differences in size between the sexes but differences in survivorship may also contribute to size distributions.

Comparisons between species indicate that male *T. ornata* and *T. carolina* mature at similar ages and female *T. ornata* and *T. carolina* mature at similar ages. In female *T.*

ornata, asymptotic size is 5 % greater than minimum size at reproduction but 17 % greater in *T. carolina*. A likely explanation is that reproductive effort (*sensu* Tinkle 1969) is greater in *T. ornata*. Evidence for this is that relative clutch mass is much greater in *T. ornata* (i. e., *T. ornata* females are much smaller but clutch mass is similar, St. Clair, in prep.). In male *T. ornata*, asymptotic size is 30 % greater than minimum size at maturity and 52 % greater in *T. carolina*. Nevertheless, interpretation of continued growth after sexual maturity must await information on size-specific mating success of males. Selection on fecundity probably favours delayed maturity in females (*T. ornata* more than *T. carolina*) and this may be more important than sexual selection on male size. Indeed, the sexes are dimorphic in other ways and size may not be critically important to male mating success. Males have more brightly colored heads (green in *T. ornata* and red in *T. carolina*) and red eyes.

The relationship between environmental sex determination and patterns of growth is inconsistent between these species. If developmental environment influenced growth rate and hence size, females should grow faster in both species and reversals of sexual size dimorphism should be due to changes in age at maturity; this is not the case. Alternatively, higher incubation temperatures may delay gonadal development rather than accelerate somatic development. The effect of incubation temperature may be separated from that of sex by hormonal manipulation of eggs to produce both sexes at a range of incubation temperatures (Rhen and Lang 1994). Subsequent observations of growth and maturation would serve to test these alternatives but would also be a formidable task in long-lived organisms.

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Appendix

If we substitute length for age in a simple exponential model of survivorship:

$$N(t) = N_0 e^{-Mt} \qquad (3)$$

where N_0 is the starting population size, N_t is the size at time t and M is the instantaneous mortality rate. If we substitute time for length (L) using the von Bertalanffy growth model then:

$$L = A(1 - e^{-Kt}) \qquad (4)$$

becomes $$t = -\frac{\ln(1 - \frac{L}{A})}{K} \qquad (5)$$

therefore number of individuals is expressed as a function of length rather than age:

$$N(L) = N_0 e^{\frac{M}{K}\ln\left(1-\frac{L}{A}\right)} \qquad (6)$$

where A is asymptotic length and K is the rate the animal approaches asymptotic length.

Tables

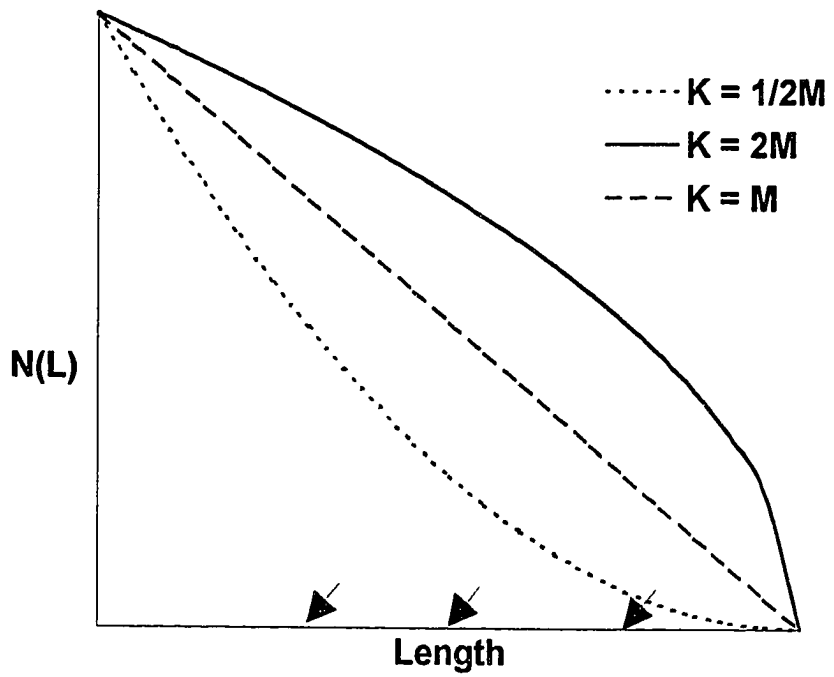
Table 1. Minimum sizes and ages at maturity versus mean size as adults.

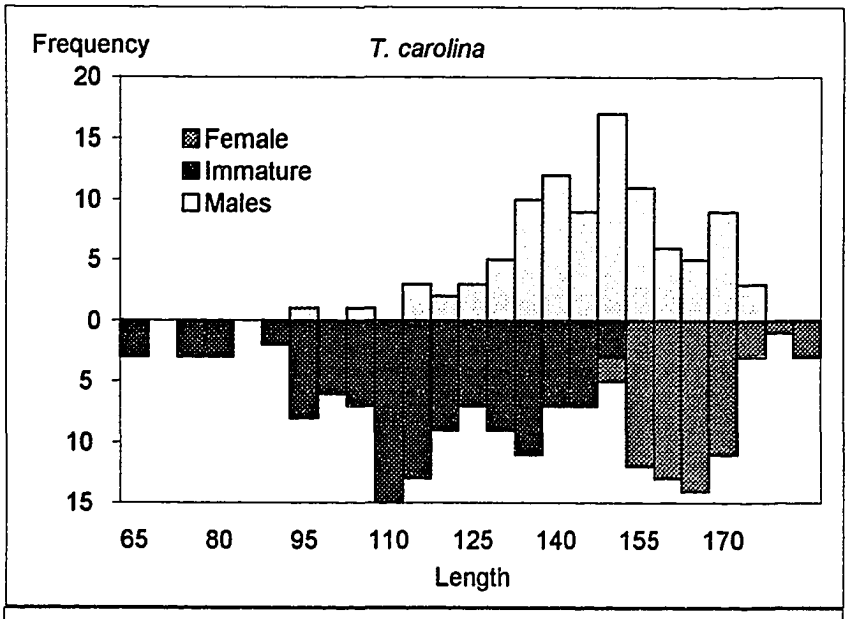
	Males		Females	
	at maturity	average	size,	average
		(± S. E., n)	age	(± S. E., n)
T. carolina	105 mm	145.6 mm	150 mm	161.9 mm
	5 yr.	(± 1.27, 100)	8 yr.	(± 1.55, 67)
T. ornata	100 mm	122.8 mm	128 mm	135.3 mm
	5 yr.	(± 1.29, 52)	8 yr.	(± 1.24, 56)

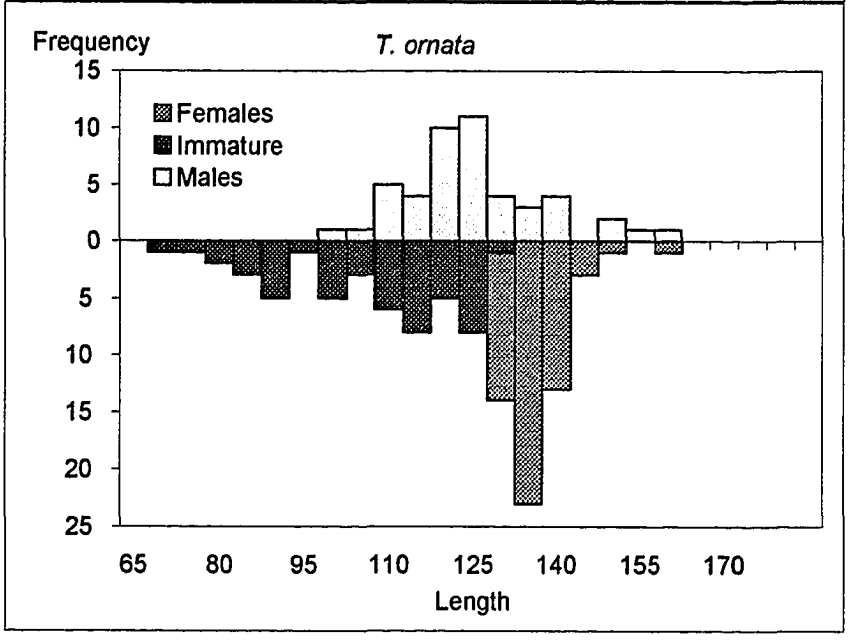
Table 2. Parameters of the von Bertalanffy growth model.

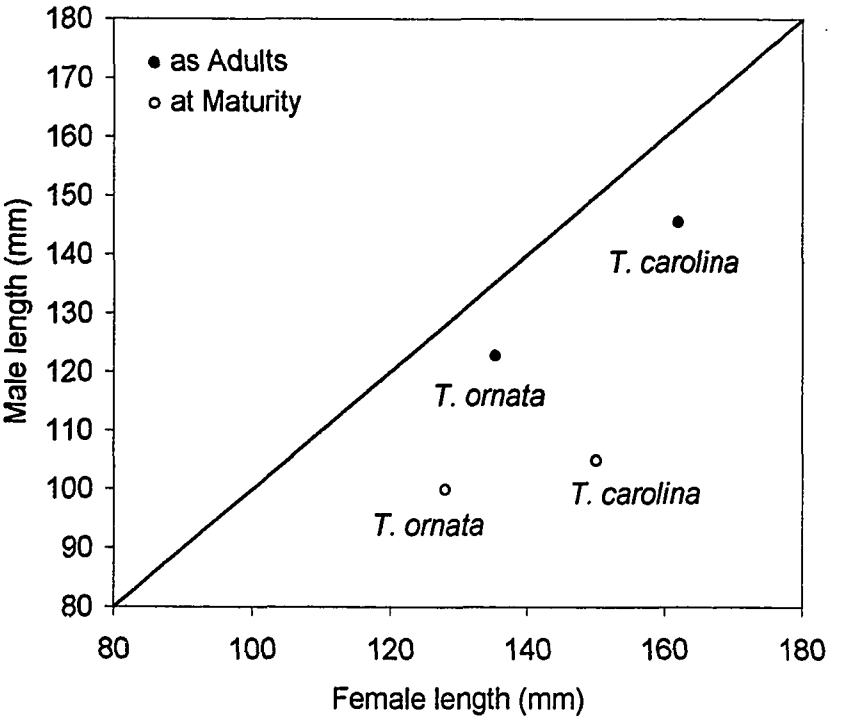
	Male		Female	
	K	Asymptote (*A*)	*K*	Asymptote (*A*)
T. carolina	0.303	160	0.210	175
T. ornata	0.346	135	0.386	130

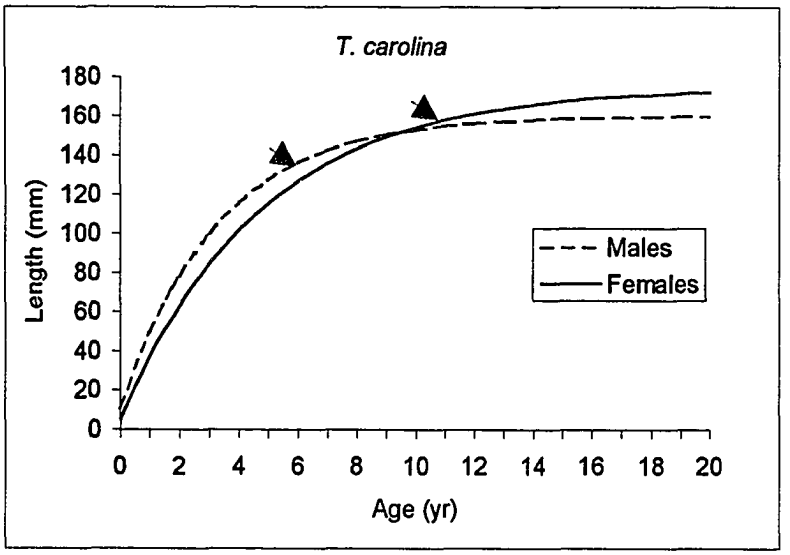
List of Figures

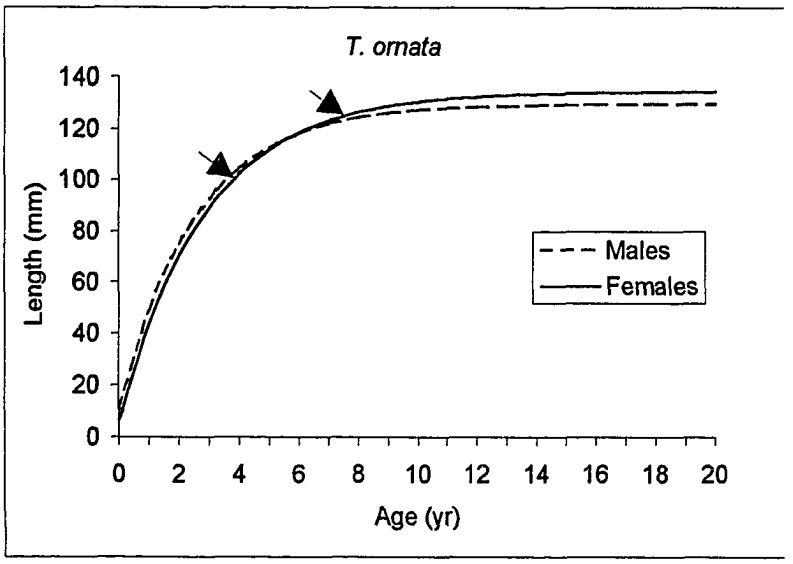


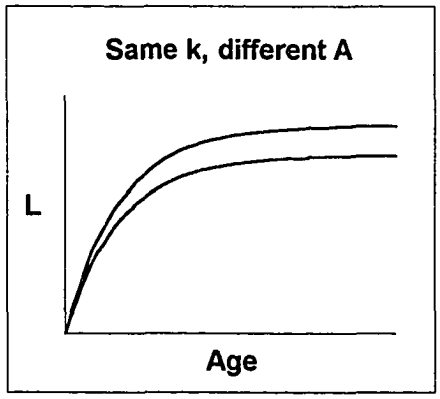


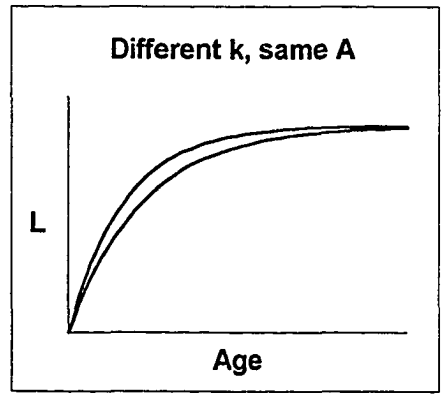


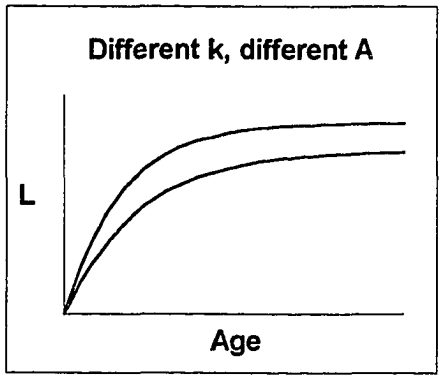


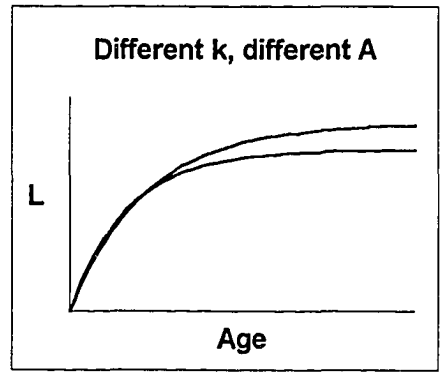












Chapter 3 . Costs of Development in Box Turtles: Temperature, Energetics, and Sex

Abstract

Developmental environment may affect survival and fecundity after hatch by modifying offspring size and condition. Where developmental environment also determines sex, early life history differences between the sexes may result. I controlled developmental temperature and measured differences in size at hatch and cost of development (in kJoules/g wet mass) in two species of box turtle, *Terrapene carolina* and *Terrapene ornata*. Eggs were incubated at 25 C and 30 C to produce males and females, respectively. In both species, hatchling wet mass was larger in males than females. Energetic cost of development was compared between the sexes by integrating oxygen consumption over development using a logistic model. Embryos incubated at lower temperatures consumed more oxygen in total than those at higher temperatures (441.9 ml. versus 426.4 ml.). Developmental costs (in kJoules per gram wet mass) were higher for males probably because they were more developed (less residual yolk). Effects of temperature on hatchling size and composition are similar to effects of hydric condition and may be because more water is incorporated into the offspring during the longer period of development at lower temperature. Because these differences at hatch are sexual in reptiles with temperature dependent sex determination, natural history observations of early life stages are necessary to interpret observations of skewed sex ratios in the wild.

Introduction

The ecology of earliest life stages is of fundamental importance because the environment in which the embryo develops affects growth and survival both during development and after hatch. Developmental environment may directly affect egg survival (Legler 1960, Bustard 1971a,b, Ferguson and Joanen 1983), hatchling morphology (Bustard 1969, Gutzke and Packard 1987a, Osgood 1978), and embryonic growth rate (Packard et al. 1977, Gutzke and Packard 1987b, Packard et al. 1987). In turn, embryonic growth rate determines time spent in the egg; this time is critically important if the egg is a vulnerable life stage (Williams 1966, Shine 1978). It is during development that the environment interacts with egg size and composition to determine size and condition of the hatchling. For example, in turtles, optimal conditions of temperature and humidity may produce the largest, healthiest offspring (see, e. g., Packard et al. 1987, Miller et al. 1987, Janzen 1993a). Assuming that offspring size and condition affect subsequent survival and growth (Ferguson and Fox 1984, Fox 1978), life history is thereby, in part, determined in the egg stage.

In some animals, developmental environment determines more than survival and growth during early life stages; it determines sex as well. Environmental sex determination is widespread, occurring in groups as diverse as nematodes and reptiles (Bull 1983, Korpelainen 1990, Janzen and Paukstis 1991a,b). It is often associated with sexual size dimorphism in which size advantages differ between the sexes, e. g., female fecundity or male competitive ability increase with size. Therefore, when developmental environment: (1) affects growth rate and size, and (2) size is more important to the

reproductive success of one sex than the other, then determination of sex by developmental environment is advantageous because it couples sex with growth rate (Charnov 1982, Bull 1983). Either the embryo can match sex with fitness differences resulting from developmental environment (see above), or the female can choose the sex of her offspring (Bull et al. 1988). Because developmental environment can affect many characters of the hatchling in addition to sex, choice of sex may involve compromising optimal developmental conditions. The description of some of these compromises is the aim of this study.

The key feature of the developmental environment that determines sex in reptiles is temperature (Pieau 1971, Yntema 1976, Bull and Vogt 1979, Ferguson and Joanen 1982). Temperature also exponentially accelerates physiological processes with the result that metabolic rate increases and incubation period is shortened (Packard et al. 1977). Metabolic rate is the rate of energy metabolism, energy which is partitioned into growth and maintenance in the developing embryo (e. g., in birds, Ricklefs 1974, Hoyt et al. 1978). The energetic cost of producing an embryo may therefore differ at different temperatures and these differences may imply different costs for producing a male versus a female offspring.

I tested proximate effects of incubation temperature on eggs and hatchlings of two species of terrestrial turtles in the genus *Terrapene*, family Emydidae: the three-toed box turtle (*Terrapene carolina triunguis*) and the ornate box turtle (*Terrapene ornata*) that have similar patterns of temperature dependent sex determination. In particular, I

compared the interaction of temperature and egg size in determining body size at hatch, embryonic growth rate, and energetic cost of development.

Material and Methods

Experimental subjects

Terrapene carolina is a primarily a woodland species and *T. ornata* is primarily a prairie species (Ernst and Barbour 1972). Gravid females were collected from the area surrounding Norman, Oklahoma, where the geographical ranges of the two species overlap. The two species have similar patterns of temperature-dependent sex determination; males are produced at 25°C and females are produced at 30°C (Ewert 1985). Number of eggs in a clutch is small (usually four) and effects of incubation environment on hatchlings have been tested only in *T. ornata* at 30 C (Packard et al. 1985).

Experimental Protocol

Oviposition was induced by injection of 0.01 cc per 100 g body mass of oxytocin (100 I.U. per ml). All eggs were assumed to be at the same stage of development because turtle eggs are suspended in the gastrula stage until laying (e. g. Ewert 1985). Experimental protocol followed Packard et al. (1987). Equal parts by mass of vermiculite and water yielded an incubation substrate at optimal hydric conditions of about -200 kPa. At laying, eggs were weighed, individually marked with pencil and placed in plastic shoe boxes, 12 to a box. Containers were placed in incubators at 25° and 30° C. Positions of

boxes were changed weekly within each incubator at which time distilled water was added. Enough distilled water was added to maintain the combined mass of box and eggs.

Incubation time

I tested effects of temperature (*T*), species (*S*), clutch (*C*), and egg mass (*m*, at laying) on incubation time (t). Clutch effects (a combination of maternal provisioning, and maternal and paternal effects) were tested using a hierarchical design of clutches nested within species. However, clutch effects were minimized because clutches were small and not more than two from each clutch were assigned to each treatment. No more than one egg from a given clutch was placed in each container. Time to hatch was compared between species and between temperatures by analysis of covariance; egg mass at laying was used as a covariate to test effects of egg size on incubation time. The linear model is as follows (μ is a constant, there were no significant interaction effects between species and incubation temperature, p = 0.596):

$$t = \mu + S + T + C + m \qquad (1)$$

Energetic cost of hatchlings

Oxygen consumption (ml/day STDP) of developing eggs was measured in a closed system with an Applied Electrochemistry Model S-3A analyzer (Sunnyvale. CA). Measurements were done in the afternoon at weekly intervals. Each egg was placed in a 50 cc syringe and returned to its incubator. The syringe was closed and, after oxygen levels had fallen by 1 - 2%, 30 cc of air from the syringe was injected into the analyzer. CO_2 and water vapor were removed (using Ascarite and Drierite, respectively) before the

sample entered the analyzer. Several blanks (empty chambers) were tested at the same time to correct for diffusion of oxygen into metabolic chambers; however, this was not a significant problem. Oxygen consumption (V) at each measurement was compared between species (S) and temperature (T) treatments by repeated measures ANOVA on oxygen consumption at each weekly sample using the following linear model, where n is the number of weeks and μ is a constant:

$$V(1..n) = \mu + S + T + S \cdot T \qquad (2)$$

I used a logistic model to describe increase in oxygen consumption over time and estimated parameters of the model using non-linear regression:

$$f(t) = \frac{K}{1 + e^{c-rt}} \qquad (3)$$

where $f(t)$ is metabolic rate (ml O_2 /h), t is time (h), K is the asymptotic metabolic rate, c is a constant that shifts the function along the t-axis, and r is the rate at which the function approaches the asymptote.

To estimate total oxygen consumed over development, I then integrated (Equation 3) over the median period of development at 25°C (75 days) and 30°C (50 days) where

$$\int_0^t f(t)dt = \frac{K}{r\left[\ln\left(1 + e^{c-rt}\right) + rt - \ln\left(1 + e^{c}\right)\right]} \qquad (4)$$

Body size at hatch

To answer the question of whether eggs incubated at 30°C and 25°C (T) produce differently-sized hatchlings (m_h) per gram of egg invested (m_e), I compared mass at hatch using egg mass at laying as the covariate in the following model, where S is species, T is temperature, C is clutch, and μ is a constant:

$$m_h = S + T + C + S \cdot T + m_e \qquad (5)$$

I then added egg mass increase (maximum mass - mass at laying) as a second covariate. Egg mass increase is the additive effect of water absorbed during development on size at hatch.

Because hatchlings incubated at different temperatures have different yolk reserves (Packard et al. 1987), I also compared mass 100 days after laying (about 50 days after hatch for eggs incubated at 30°C and about 25 days after hatch for eggs incubated at 25°C). At this time hatchlings had started to eat, an indication that yolk reserves had been absorbed.

Statistics

Only hatchlings that survived for one month past hatch were used in the analyses (*T. carolina*: 34 eggs from 13 clutches, *T. ornata*: 21 eggs from 7 clutches). General linear models (Wilkinson 1990) were used to compare main effects and interaction effects. Test statistics for mixed and random effects models were calculated as in Neter et al. (1985, p. 788). The assumption of homogeneity of slopes in analysis of covariance was

tested by examining the interaction between the covariate and main effects. All summary statistics are means and standard errors unless otherwise noted.

Results

Incubation Time

There was no significant effect of egg mass at laying ($F_{1,52} = 0.32$, $p = 0.572$) or clutch ($F_{23,52} = 0.57$, $p = 0.362$) on incubation time. There was a significant effect of species ($F_{1,52} = 5.43$, $p = 0.024$) and incubation temperature ($F_{1,52} = 275.37$, $p < 0.001$) on incubation time. There was a significant increase (Tukey's HSD, $p < .001$) in incubation time at 25°C (Fig. 1, 26-day increase, i. e., 51%). Species differed at 30° in that *T. carolina* eggs took on average 2.7 days (5%) longer to hatch than did *T. ornata* (Tukey's HSD, $p = 0.018$); there was no difference between species at 25° (Tukey's HSD, $p = 0.922$).

Energetic cost of hatchlings

There was no difference in rates of oxygen consumption between species at either 25°C ($p = 0.937$) or at 30°C ($p = 0.736$). There were (not surprisingly) significantly higher rates of oxygen consumption at 30° compared with 25° ($p < 0.001$).

The estimated logistic functions were:

$$25°: f(t) = \frac{22.685}{1 + e^{7.16 - 0.14t}}$$

$$30°: f(t) = \frac{22.798}{1 + e^{6.25 - 0.20t}}$$

Asymptotic metabolic rates (K) and constants (c) did not differ between incubation temperatures but rates of increase in metabolic rates (r) did (Fig. 2). The similarity between asymptotic metabolic rates is confusing because metabolic rates should be higher at higher temperature. However, unexpectedly high measurements of metabolic rate at low temperature may occur because proportion of metabolically active tissue is higher in those embryos (see below). Because measurements were repeated on the same eggs, F-tests of significant differences among parameters are not reported although estimates of the parameters will be accurate (Horton 1978). Similarly, confidence limits cannot be estimated for integrals under the logistic curves. The total oxygen consumed over development was 422.9 ml at 30°C and 536.9 ml at 25°C. This translates to 8.31 kJ and 10.55 kJ, where 1 J = 19.64 ml O_2 (Vleck et al. 1984), a 24% increase for slower-developing embryos.

Body size at hatch

There was a linear increase in hatchling size with egg size in all cases (Fig. 3, $p < 0.001$; *T. carolina* 30°: $r^2 = 0.90$, n = 13, *T. carolina* 25°: $r^2 = 0.86$, n = 14, *T. ornata* 30°: $r^2 = 0.88$, n = 10, *T. ornata* 25°: $r^2 = 0.80$, n = 9). There was a difference between slopes in *T. ornata* ($p = 0.146$). When corrected for egg mass at laying, there was no difference in hatchling mass between species ($p = 0.414$); hatchlings incubated at 25° C were larger for both species ($p = 0.001$, Table 1). Hatchling mass was significantly greater for *T. carolina* hatchlings incubated at 25° C. In *T. ornata*, although the slopes of the relationship between egg mass at laying and hatchling mass are statistically indistinguishable, the differences in the effect of egg mass on hatchling mass at different

temperatures seem to lessen at higher egg mass (Fig. 3). In fact, much of the variance in hatchling size could be accounted for by water absorbed during development rather than temperature alone; egg mass alone accounted for 82% of the variance ($p < 0.001$), egg mass + mass increase ($p < 0.001$) accounted for 90%, and egg mass + mass increase + temperature ($p = 0.034$) accounted for 91%. There was no interaction between mass gain and temperature ($p = 0.605$) and therefore no consistent bias in mass gain at either temperature treatment. Therefore, although I attempted to maintain water potential throughout incubation, individual differences in water absorption (along with egg size at laying) accounted for most of the variance in hatchling size (Table 1).

In both species, clutch had a significant effect on hatchling size ($p = 0.001$). However, the effect of clutch on hatchling size was due to the close association between clutch and egg mass. The previous effect of egg mass on hatchling mass became insignificant when clutch was introduced into the model. I used an ANOVA to test differences in egg mass among clutches; there were significant differences among clutches and much of the variance in egg mass could be explained by clutch alone (*Terrapene carolina*: $r^2 = 0.88$, $p < 0.001$, *Terrapene ornata*: $r^2 = 0.84$, $p < 0.001$).

There was a linear increase in hatchling size with egg size in all cases ($p < 0.001$; *T. carolina* 30°: $r^2 = 0.90$, $n = 13$, *T. carolina* 25°: $r^2 = 0.86$, $n = 14$, *T. ornata* 30°: $r^2 = 0.88$, $n = 10$, *T. ornata* 25°: $r^2 = 0.80$, $n = 9$). There was a difference between slopes in *T. ornata* ($p = 0.146$). Hatchling mass was significantly greater for *T. carolina* hatchlings incubated at 25° C.

After hatchlings had absorbed residual yolk and begun to eat (approximately 6 weeks after hatch for eggs incubated at 30 C and 2 weeks after hatch for eggs incubated at 25 C), egg mass no longer correlated with size of hatchlings that had been incubated at 30° (*Terrapene carolina*: $p = 0.666$, *Terrapene ornata*: $p = 0.389$) but still did affect size of hatchlings that had been incubated at 25° (*Terrapene carolina*: $r^2 = 0.77$, $p < 0.001$, *Terrapene ornata*: $r^2 = 0.73$, $p = 0.003$).

Discussion

At the lower incubation temperature, time spent in the egg was increased by 26 days (51%), energy input necessary to produce an offspring was increased by 2.24 kJ (24%), and hatchlings were very slightly larger (0.25 g, 3.2%). Because lower incubation temperatures also produce males, these are differences between females and males at the point of hatch. Although differences are inevitably associated with sex, there is evidence that it is temperature rather than sex that is responsible. For example, Rhen and Lang (1994) separated the effects of sex and temperature in snapping turtles by hormonally producing each sex at a range of temperatures; they found that growth and size were affected by temperature, not sex.

Proximate effects of higher incubation temperature on energetic costs of development include faster development (Q_{10} effects) and less complete development at hatch. By less complete development, I mean that a higher proportion of the body mass is residual yolk (Packard et al. 1987, Packard et al. 1988). The reason for the relatively larger store of lipid in hatchlings incubated at higher temperature is unknown but it seems

to be coupled with accelerated development. Lower water potential during development seems to prolong development in a manner similar to higher temperature (Packard et al. 1983, Packard et al. 1988). Higher water potential increases hatchling size (Gettinger et al. 1984, Packard et al. 1983, Packard et al. 1985, Packard et al. 1987, Packard and Packard 1989) and, although I followed the Packard et al. (1987) protocol, I found that individual differences in amount of water absorbed during development had a stronger effect on hatchling size than did temperature (cf. Cagle et al. 1993). Lower incubation temperature results in larger hatchlings in many reptiles (Table 2) and larger mass may be due to prolonged development during which time more water is absorbed and incorporated into the embryo. Evidence for the importance of water absorption comes from turtles with rigid-shells (Table 2). One species (*Trionyx triunguis*) with rigid-shelled eggs and without environmental sex determination shows no difference in size at hatch with different incubation temperatures (Leshem et al. 1991) and another within the same genus (*Apalone mutica*, previously *Trionyx*) shows the opposite pattern, larger size at hatch at higher temperature (Janzen 1993b). Except *Chelydra serpentina*, among reptiles in Table 2 with environmental sex determination, the larger sex is produced at higher temperature.

Reducing time spent in the egg may be advantageous because this is the most vulnerable life stage in turtles (e. g. Cagle 1950, Wilbur 1975, Crouse et al. 1987, Frazer et al. 1990, Frazer et al. 1991). However, extending this period may not be a problem because nests are most vulnerable to predation during the first few days (Tinkle et al. 1981, Congdon et al. 1983, 1987, Christens and Bider 1987). In addition, regardless of

when they hatch, hatchlings of many species remain in the nest over the winter and emerge in the spring (Gibbons and Nelson 1978, Breitenbach et al. 1984, St. Clair and Gregory 1990, Lindeman 1991). Therefore, in those species that overwinter in the nest, hatchlings would seem to be trading time spent in the nest for time spent above ground (*sensu* Shine 1978, "safe harbor hypothesis"). However, some turtle species with environmental sex determination do not overwinter in the nest (e. g. *Chelydra serpentina* and *Sternotherus odoratus*, Gibbons and Nelson 1978) and because incubation temperature determines both sex and incubation period, a sex bias in time of emergence is likely; those hatchlings that emerge earlier may encounter different perils than those that emerge later. Unfortunately, patterns of emergence are unknown in *Terrapene*. The connection between sex and emergence in *C. serpentina* and *S. odoratus* is further complicated because females develop at both high and low temperatures (Yntema 1976, Vogt at al. 1982). In the north, because eggs in the nest (in those species that emerge in the spring) and hatchlings in the nest (in those species that emerge in the fall) cannot survive over the winter, it still may be dangerous to delay hatching (Schwarzkopf and Brooks 1987, Bobyn and Brooks 1994a).

The assumptions are that larger hatchlings survive better and residual yolk may be of advantage in surviving the winter in the north. However, these assumptions are poorly documented. The best evidence for survival advantages of larger size to reptile hatchlings comes from two studies on lizards by Sinervo et al. (1992) and Ferguson and Fox (1984). The study by Sinervo et al. (1992) is noteworthy because it demonstrates differences in size advantages between males and females. The best evidence for turtles comes from

Janzen (1993a), who used hydric conditions during development to produce a range of offspring sizes and demonstrated higher survivorship for larger offspring. However, apart from size, low hydric conditions *per se* disadvantage offspring (Miller at al. 1987). Tests in reptiles of the effects of residual yolk on survival over the winter are rare, but at least one study (on rattlesnakes) has failed to detect an effect of offspring mass on survivorship over the winter (Charland 1989).

The fact that female hatchlings hatch sooner, with more residual yolk, and at a smaller size may generate sexual asymmetries in early survival of offspring in turtle species with environmental sex determination. This leads to questions that could be answered with more information on the natural history of turtle hatchlings. Among wild-caught animals, sex ratio in *T. carolina* was skewed towards males (99:65, $\chi^2 = 4.88$, $p = 0.059$) and, in *T. ornata*, slightly more females than males (58:51, $\chi^2 = 0.22$, $p = 0.64$). This may be consistent with environmental differences if nests in grasslands are hotter than nests in woodlands. Nevertheless, effects on sex ratio of unequal thermal distribution of incubation sites must be separated from differences in survivorship. Regardless, effects of survivorship on sex ratio may be irrelevant to selection for environmental sex determination because mortality occurs after parental investment (Fisher 1958). The reduction in parental fitness due to the production of the sex that suffers higher mortality is exactly balanced by the fitness gained by producing the scarcer sex.

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Tables

Table 1. Effects of incubation temperature on hatchling mass.

Species	T (°C)	mean ± SE - adjusted for egg mass at laying	mean ± SE - adjusted for maximum egg mass	n
T. c. triunguis	30	7.760 ± 0.088	7.834 ± 0.070	15
	25	8.076 ± 0.081	8.039 ± 0.064	18
T. ornata	30	7.895 ± 0.129	7.932 ± 0.101	7
	25	8.088 ± 0.104	8.023 ± 0.083	11

Table 2. Hatchling size and incubation temperature in reptiles. Letters in parentheses refer to taxonomic group: T = turtle, S = snake, L = lizard, C = crocodile, Tu = Tuatara

Temperature Effect	Species	Egg shell	Sex determination	Ref.
Larger at lower	*Chelydra serpentina*(T)	flexible	environment	1,2,3,4
incubation temperature	*Emydoidea blandingii*(T)	flexible	environmental	5
	Carettochelys insculpta(T)	rigid	environmental	6
	Chrysemys picta(T)	flexible	environmental	7
	Pituophis melanoleucus(S)	flexible	genetic	8
	Iguana iguana(L)	flexible	genetic	9
	Podarcis muralis(L)	flexible	genetic	10
	Crocodylus johnstoni(C)	flexible	environmental	11,12
	Crocodylus porosus(C)	flexible	environmental	13
	Alligator mississipiensis(C)	flexible	environmental	14
	Terrapene carolina(T)	flexible	environmental	15
	Terrapene ornata(T)	flexible	environmental	16
No effect of temperature	*Trionyx triunguis*(T)	rigid	genetic	17
	Malaclemys terrapin(T)	flexible	environmental	18
	Alligator mississipiensis(C)	flexible	environmental	19
	Crocodylus niloticus(C)	flexible	environmental	20
	Sphenodon punctatus(Tu)	flexible	genetic?	21
Smaller at lower	*Apalone mutica*(T)	rigid	genetic	22
incubation temperature	*Pituophis melanoleucus*(S)	flexible	genetic	23

[1] Packard et al. 1987, 1988; [2] McKnight and Gutzke 1993; [3] Brooks et al. 1991 (except at very low temperatures); [4] Bobyn and Brooks 1994b (effect at dry substrate only); [5] Gutzke and Packard 1987; [6] Webb et al. 1987; [7] Gutzke et al. 1987; [8] Gutzke and Packard 1987b; [9] Phillips et al. 1990; [10] Van Damme et al. 1992; [11] Webb et al. 1987; [12] Whitehead et al. 1986; [13] Webb et al. 1987; [14] Deeming and Ferguson 1989; [15] This study; [16] This study; [17] Leshem et al. 1991; [18] Roosenburg and Kelley, in press; [19] Joanen et al. 1987; [20] Hutton 1987; [21] Thompson 1990; [22] Janzen 1993b; [23] Burger et al. 1987

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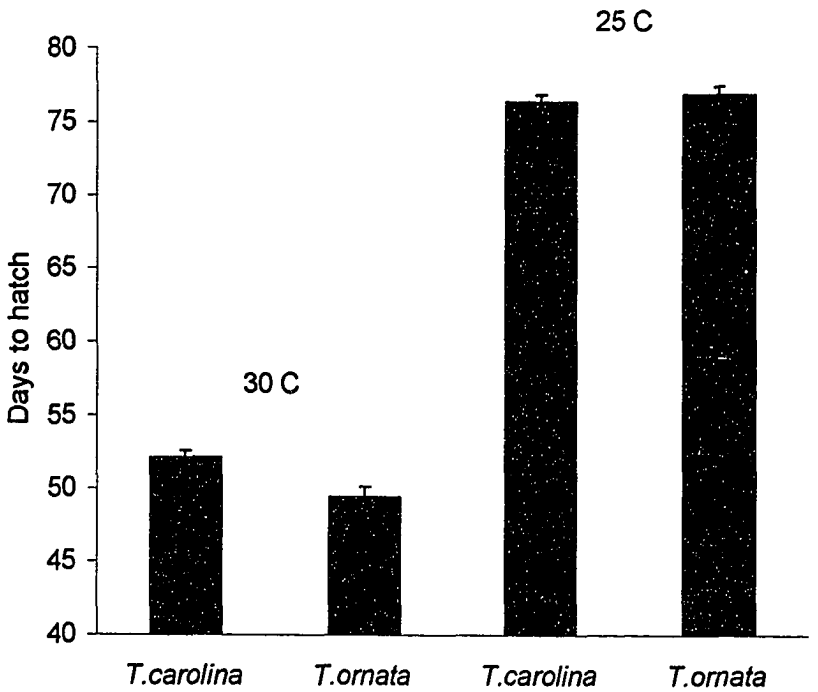

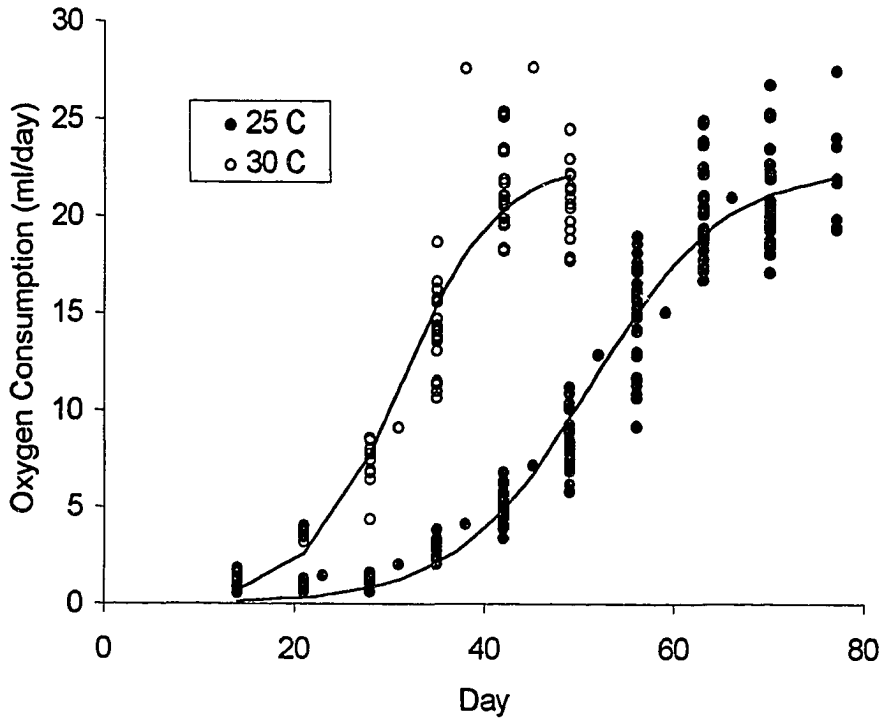

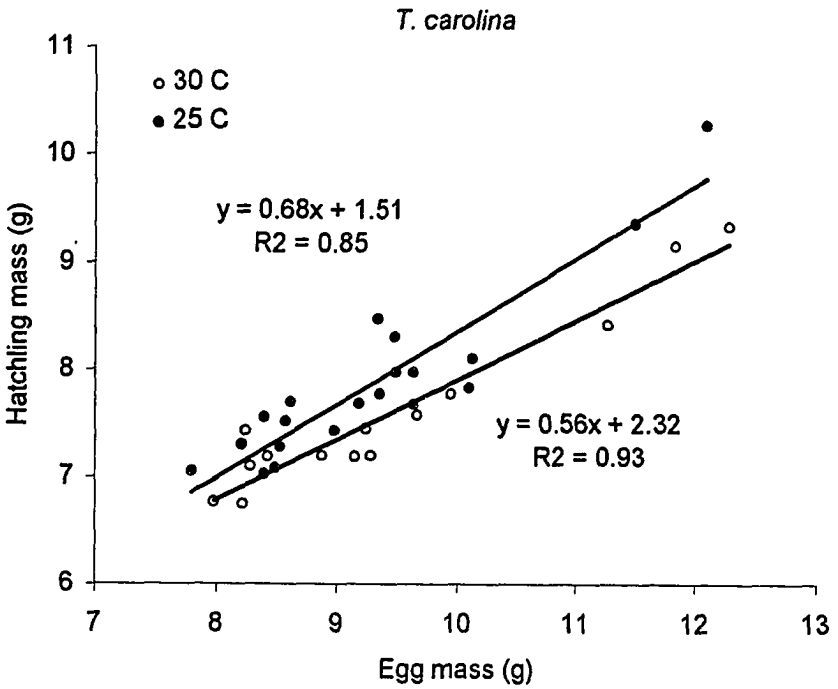

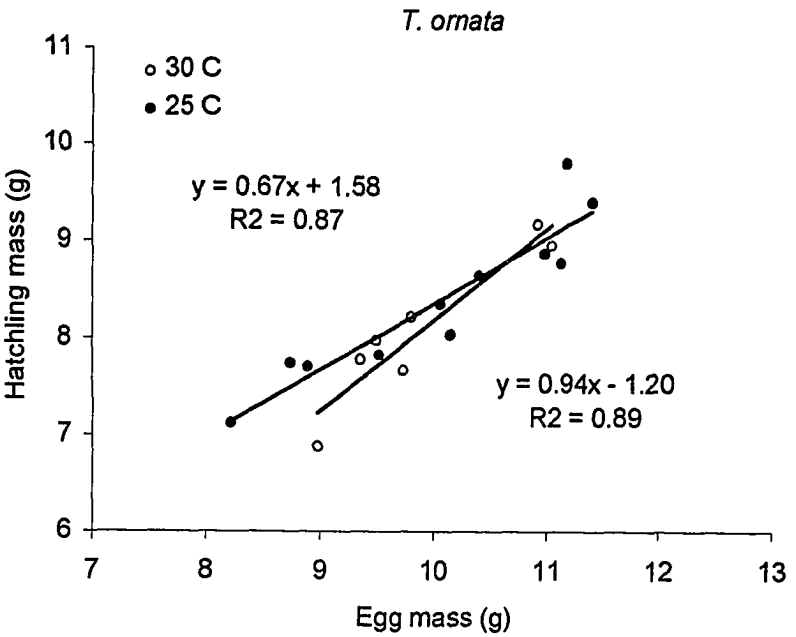

Chapter 4 . Post-hatch effects of incubation temperature on growth and metabolic rate in box turtles (*Terrapene*).

Abstract

Developmental temperature may influence subsequent life history by modifying offspring size, condition, and growth. Consequently, in animals with temperature dependent sex determination, life history is coupled with sex . Here I describe differences in growth and metabolic rate in hatchlings of two species of box turtle, *Terrapene carolina* and *Terrapene ornata*. Eggs were incubated at 25 C and 30 C to produce males and females, respectively. Hatchlings were then raised under common conditions. After a year and a half, growth did not differ between the sexes although growth differed between species. Metabolic rate at 20 C, 25 C, and 30 C differed significantly between the sexes but not between species. Among these three temperatures, metabolic rate was highest at the temperature the animal had experienced as a hatchling; i. e., males at 25 C and females at 30 C. This difference among metabolic rates provides a mechanism by which growth rates could differ between the sexes in the wild, despite there being no observed difference under laboratory conditions.

Introduction

In some animals, developmental environment determines hatchling sex (Charnov and Bull 1977). If developmental environment also determines some aspect of fitness and, furthermore, if these differences in fitness are biased by sex, then it is beneficial to couple developmental environment with sex determination. For example, if large size advantages females more than males (*sensu* Ghiselin 1969) and higher incubation temperature is

positively associated with size at maturity, then associating higher incubation temperature with female sex is advantageous. An association between size, sex, and incubation temperature is found in two clades of vertebrates with environmental sex determination, crocodiles and turtles. In general, the larger sex comes from warmer nests. The comparison between turtles and crocodiles is provocative because patterns of sexual size dimorphism match patterns of temperature-dependent sex determination and are opposite in the two groups (Head et al. 1987). Male crocodiles are larger, grow faster (Lang 1985), and come from warmer nests; in contrast, female turtles come from warmer nests and, in many species, are larger than males. In crocodiles, the adaptive explanation for sexual size dimorphism is that males compete for mates and guard territories; therefore males benefit more from larger size than do females (Head et al. 1987). In most turtles there may be no sexual selection on male size and females may benefit more from large size because larger females produce more or larger offspring (Berry and Shine 1980). However, the observations of Head et al. (1987) beg two questions: how does warmer nest temperature contribute to size at maturity? and, what are the advantages to size in one sex versus the other? In this study, I explore the former question using growth rate as a possible link between incubation temperature and sexual size dimorphism.

In turtles, any effect of incubation temperature on size is inconsistent among different species because, even though females still come from warmer nests, there are some species in which males are, on average, larger than females (Janzen and Paukstis 1991). In these species in which males are larger, either males grow more slowly but are larger because they delay maturity, or they grow faster, hence growth after hatch is not correlated with lower incubation temperature (contra Head et al. 1987). To separate these

alternatives, I tested proximate effects of incubation temperature on eggs and hatchlings of two species of box turtles (the three-toed box turtle, *Terrapene carolina triunguis* and the ornate box turtle, *Terrapene ornata*) that were reported to have different patterns of sexual size dimorphism (Janzen and Paukstis 1991) although this does not seem to be the case in my study population (St. Clair, in prep.). Incubation temperatures were chosen to produce either males or females. In particular, I compared growth rate of hatchlings that had been incubated at high and low temperatures and metabolic response of these hatchlings to changes in temperature. I measured metabolic rate because it may be a link between growth and incubation temperature; for example, incubation temperature may affect thermoregulation of hatchlings (Lang 1985, in crocodiles) which, in turn, may influence growth rate.

Material and Methods

Experimental subjects

Box turtles are a terrestrial genus (*Terrapene*) in the family Emydidae. *T. carolina* is found primarily in woodlands and *T. ornata* is found primarily in prairies (Ernst and Barbour 1972, pers. obs.) but their geographical ranges overlap in Norman, Oklahoma, where I collected gravid females in June, 1993. Number of eggs in a clutch is small (usually four) and effects of incubation environment on hatchlings have previously been tested only in *T. ornata* and only at 30 C (Packard et al. 1985). The two species have similar patterns of temperature-dependent sex determination; males are produced at 25°C and females are produced at 30°C (Ewert 1985). Although the two species have similar

patterns of environmental sex determination, adult males are larger, on average, than adult females in *T. carolina* and equal in size to females in *T. ornata* (Janzen and Paukstis 1991) although this turned out not to be the case in the turtles I collected.

Incubation Protocol

Oviposition was induced by injection of 0.01 cc per 100 g body mass of oxytocin (100 I.U. per ml). All eggs were assumed to be at the same stage of development because turtle eggs are suspended in the gastrula stage until laying (Ewert 1985). Experimental protocol followed Packard et al. (1987); equal parts by mass of vermiculite and distilled water yielded an incubation substrate at optimal hydric conditions of about -200 kPa. At laying, eggs were weighed, individually marked with pencil, and placed in plastic shoe boxes, 12 to a box. No more than one egg from each clutch was placed in each container. Incubators were maintained at 25° and 30° C. Positions of boxes were changed weekly within each incubator and distilled water was added to maintain the original water potential. Because hatchlings were to be used to test differences in growth, sex was confirmed only in those that died from mishap after hatch (n = 7). In all of these cases, hatchlings were the sex predicted from incubation temperature (Ewert 1985).

Growth rate of hatchlings

I placed 5 - 7 hatchlings in cages (50 x 70 cm) and fed them *ad libitum* on Purina trout chow. A single 100 W light above each cage provided heat. Individuals from both temperature treatments were mingled and each week assigned by chance to cages. Mass of hatchlings (m) was measured at hatch and after 584 days and compared by species (S),

incubation temperature (T), and clutch (C) using ANCOVA with mass at hatch (m_0) as the covariate. The effect of clutch (C) is nested within species, μ is a constant:

$$m = \mu + S + T + S \times T + C + m_0 \qquad (1)$$

Clutch effect is a mixture of egg size (maternal provisioning), and maternal and paternal genetic effects.

Metabolic rate of hatchlings

After 10 months, I tested the effects of incubation temperature on metabolic rate (oxygen consumption) of hatchlings at 20°C, 25°C, and 30°C. I measured oxygen consumption (ml/day STDP) with a closed system (Applied Electrochemistry Model S-3A analyzer, Sunnyvale. CA). Measurements were done in the afternoon at weekly intervals. Hatchlings were held at each test temperature for one week and starved for the four days prior to testing. Hatchlings were placed in closed respirometers which were then returned to the incubator. A sample was removed and injected into the analyzer after removal of CO_2 (using Ascarite) and water vapor (using Drierite). Trial times were adjusted so that oxygen levels fell by about 1 - 2%. Several empty chambers were tested at the same time to correct for diffusion of oxygen into metabolic chambers; however, this was not a problem. Mass specific metabolic rate (MR) was compared between species (S) and incubation temperatures (T) by repeated measures ANOVA using the following model where μ is a constant:

$$MR_{20,25,30} = \mu + S + T + S \cdot T \qquad (2)$$

Statistics

General linear models (Wilkinson 1990) were used to compare main effects and covariate effects. The assumption of homogeneity of slopes in analysis of covariance was tested by examining the interaction between the covariate and main effects. All summary statistics are means and standard errors unless otherwise noted.

Results

Growth rate of hatchlings

Terrapene carolina hatchlings grew significantly faster then *T. ornata* hatchlings ($p = 0.037$, Fig. 1) but there was no effect of incubation temperature on subsequent size ($p = 0.851$). However, there was a plausible interaction between incubation temperature and species ($p = 0.150$, *T. carolina* males grew faster than females and *T. ornata* females grew faster than males) so the analysis was repeated separately for each species. There was no significant difference in growth between the two temperature treatments (*T. carolina*: $p = 0.368$, *T. ornata*: $p = 0.561$).

Neither clutch ($p = 0.302$) nor mass at hatch ($p = 0.809$) had a significant effect on hatchling growth. However, these two variables are confounded because clutch is significantly related to egg mass and mass at hatch ($p < 0.001$). As a consequence, mass at hatch had an effect on growth ($p = 0.051$) if clutch was removed from the model and vice-versa (although not significant, $p = 0.114$). The persistent effect of mass at hatch on subsequent hatchling mass was confined to *T. carolina* ($p = 0.046$).

Metabolic rate of hatchlings

There was a difference in metabolic rates between incubation treatments ($p = 0.025$) but no difference between species ($p = 0.438$). Metabolic rates of hatchlings that had been incubated at 30°C increased linearly throughout the temperature treatments. There was a quadratic (second degree polynomial) effect in hatchlings that had been incubated at 25°C; metabolic rates increased to 25°C and then decreased at 30°C (Fig. 2).

Discussion

In *Terrapene* hatchlings raised under common conditions, growth was not affected by the temperature they had experienced as embryos. However, holding animals under common conditions ("common garden" approach) does not mean that conditions did not favor either sex or either species. For example, *T. carolina* hatchlings grew faster but they might not under a different experimental regime (i. e., a different "garden"). The only effect on growth rate detectable after almost 2 years under common conditions was size at hatch, and that occurred only in one treatment, *T. carolina* incubated at 25°C. This contrasts with the turtle, *Malaclemys terrapin* (Roosenburg and Kelley, in press), in which hatchlings from larger eggs incubated at 32°C were larger at the end of three years whereas egg size had no lasting effect on hatchlings incubated at 26°C. Hatchlings from eggs incubated at 32°C also grew faster than those incubated at 26°C (Table 1). The interaction of growth rate and egg size suggests that adult females could exaggerate the effects of large egg size on hatchling size by placing larger eggs in higher temperature

nests. This seems to be the case in *Malaclemys* because, on average, larger eggs are placed in warmer locations (Roosenburg, in press).

Among those reptiles tested, growth rate of hatchlings is affected by developmental temperature (Table 1), but this effect is inconsistent; higher temperature does not necessarily mean faster growth (e. g. *Chelydra serpentina*). In *C. serpentina,* growth rate is higher at intermediate temperatures and may be due to interaction between sex hormones and developmental temperature. However, Rhen and Lang (1994) controlled for the effect of sex by hormonally producing both sexes at three temperatures; growth rates were affected by incubation temperature, not sex. Although the effect of incubation temperature varies among species, growth rate of hatchlings does seem to match patterns of adult sexual size dimorphism (Table 1). Nevertheless, although size partly depends on growth rate, size also depends on duration of growth (age at maturity). In turtles, females often mature later than males (review in Bury 1979) and hence, regardless of growth rate, may be larger because they grow for a longer period of time.

Adaptive explanations for temperature dependent sex determination depend on: (1) some link between adult fitness and developmental temperature and (2) asymmetries between the sexes in fitness benefits associated with developmental temperature (Charnov and Bull 1977, Head et al. 1987). The simplest scenario is that size benefits one sex more than the other and developmental temperature affects size; therefore, linking developmental temperature to sex is beneficial. However, the link does not seem to consist simply of higher temperature being associated with faster growth. The link between developmental temperature and size may not be growth rate at all. For example,

developmental temperature may affect gonadal development and, hence, timing of sexual maturity. Alternatively, developmental temperature may only be a reliable indicator of some other factor that promotes adult size. Developmental temperature would therefore still be a useful secondary cue for determining sex of the embryo.

In conclusion, although there was no difference in growth between the sexes raised under laboratory conditions, I found that metabolic rates of hatchlings were highest at the temperature at which the hatchling had been incubated. This suggests different temperature optima (*sensu* Huey 1982) for metabolic rates in males and females and provides a physiological connection between growth and thermal selection, assuming that animals that select higher temperatures grow faster. Although these tests should be replicated at later life stages, they suggest a mechanism for sexual differences in growth.

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Tables

Table 1. Effects of incubation temperature on hatchling growth in reptiles.

Temperature effects	Species	Sexual size dimorphism	Temperature	Ref.
High > low	*Malaclemys terrapin*	Females > males	High = females	1
Intermediate > high > low	*Alligator mississippiensis*	Males > females	High = males	2
High > low	*Crocodylus niloticus*	Males > females	High = males	3
Intermediate > (low = high)	*Chelydra serpentina*	Males > females	High, low = females	3
(intermediate = low) > high	*Chelydra serpentina*	Males > females	High, low = females	4

1 Roosenburg and Kelley (in press); [2] Joanen, McNease, and Ferguson (1987); [3] Hutton (1987); McKnight and Gutzke (1993); [4] Rhen and Lang (1994)

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