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Environmental effects on fitness and consequences for sex allocation in a reptile with environmental sex determination

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ABSTRACT

Although populations of most sexual species exhibit a 1 : 1 sex ratio, biased ratios are often associated with environmental sex determination. Theoretical models predict that species with environmental sex determination will bias sex allocation in favour of the sex produced in the environment that yields lower fitness. These models have been proposed as an explanation for the biased sex ratios observed in many reptiles with environmental sex determination. We explore the effects of developmental environment on sex allocation in *Graptemys ouachitensis*, a turtle with environmental sex determination. We found that developmental environments producing males were poorest for survival, growth and performance, suggesting that the sex ratio of *G. ouachitensis* should be male-biased. This expectation contrasts with the observed female bias in *G. ouachitensis*. Warmer than average temperatures may have contributed to the female-biased hatchling sex ratios reported for *G. ouachitensis* in some studies. However, the strongly skewed population sex ratios in map turtles indicate female-biased allocation. The frequent finding of female-biased primary sex ratios in many species indicates an overall trend of female-biased sex allocation in reptiles with environmental sex determination. The conflict between this tendency for female biases and our results suggests that environmental effects on fitness will not explain the general tendency to skewed sex ratios. The apparent incongruency between our findings and theoretical models concerning sex allocation and environmental sex determination suggests that an alternative model that focuses on nest-site inheritance might explain better the female-biased sex ratios observed for *G. ouachitensis* and other reptiles with environmental sex determination.

Keywords: *Chelydra serpentina*, *Graptemys ouachitensis*, nest-site inheritance, sex ratio, temperature-dependent sex determination.

INTRODUCTION

Fisher (1930) predicted that populations should exhibit 1 : 1 sex ratios when the cost of each sex is equal, due to frequency-dependent selection for the minority sex. Although most natural populations do exhibit sex ratios near unity, certain factors are known to result in skewed population sex ratios in natural systems. Theoreticians have elucidated several forces

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known to produce biased sex ratios, and empirical investigations have supported these predictions (reviewed in Charnov, 1982; Bull and Charnov, 1988). A strong trend towards female biases in reptiles with environmental sex determination has been reported (Bull and Charnov, 1988; Ewert and Nelson, 1991), but theoretical models that might explain this pattern have not been tested previously.

Primary and secondary sex ratios in reptiles with environmental sex determination typically range from even to female-biased (Bull and Charnov, 1988; Ewert and Nelson, 1991). Extreme female biases – greater than 10 females per male – are known from at least six natural populations of four species (reviewed in Woodward and Murray, 1993; Freedberg and Wade, 2001). Furthermore, when male biases have been found, these have pertained to adult sex ratios that are often explainable by sex differences in post-hatching life history and, therefore, are consistent with classical Fisherian theory (Lovich and Gibbons, 1990; Lance *et al.*, 2000). A predominance of female-biased primary sex ratios in reptiles with environmental sex determination is puzzling, as Fisherian theory predicts the female biases should result in greater relative fitness for males, driving the sex ratio back to unity.

The frequent finding of female-skewed sex ratios in reptiles has prompted several explanatory hypotheses, including differences in mortality between the sexes (Lance *et al.*, 2000), migration (Morreale *et al.*, 1982) and age to maturity (Lovich and Gibbons, 1990), as well as environmental effects on fitness (Bull and Charnov, 1989) and cultural inheritance of nest site (Freedberg and Wade, 2001). Of these explanations, only the last two models are applicable to biased primary sex ratios. Models that explain the primary sex-ratio bias in other systems are not consistent with the known biology of reptiles with environmental sex determination. For instance, cytoplasmic sex determination (Shaw, 1958) is inconsistent with incubation temperatures that produce exclusively males, while local mate competition (Hamilton, 1967) does not seem plausible in most reptiles with environmental sex determination, although it is compatible with the biology of a snake that lacks such sex determination (Madsen and Shine, 1992).

Sex-ratio bias through environmental effects on fitness has been predicted by several models (Bull, 1981; Frank and Swingland, 1988; Charnov and Bull, 1989a,b). Each of these assumes that the developmental environment differentially affects the lifetime fitness of the sexes in species with environmental sex determination. Specifically, these models assume that individuals produced in ‘good’ environments have relatively high lifetime fitness, whereas individuals produced in ‘poor’ environments have negligible reproductive value. If the poor environment produces predominantly or exclusively one sex, then selection will favour a sex ratio close to unity in the good environment. This will result in a primary sex ratio that favours the sex produced in the poor environment, since these low-quality individuals can accumulate without countervailing selection for the minority sex. The primary sex ratio will be equal to the ratio of lifetime fitness resulting from male incubation temperatures to the fitness resulting from incubation at female-producing temperatures (Bull and Charnov, 1989).

Environmental sex determination thus provides an opportunity for fitness differences between the sexes to result in biased sex allocation, while genetic sex determination does not (Appendix 1). These models imply that ‘poor’ environments are unavoidable, such that a fraction of offspring develop there. In reptiles, suboptimal nesting environments are most likely to result from seasonal and year-to-year fluctuations in ambient temperature and precipitation.

If the female-biased sex ratios are indeed a result of fitness differences associated with incubation environment, these models predict that, within the range of naturally occurring nest temperatures, temperatures that produce female offspring should have lower fitness (Fig. 1). Several studies have explored the effects of incubation environment on components of post-hatching phenotype in reptiles with environmental sex determination (Congdon *et al.*, 1995; Rhen and Lang, 1995; Janzen *et al.*, 2000; for a review, see Shine, 1999). However, these experiments did not incorporate a full range of natural nest temperatures, preventing clear inferences about the factors shaping natural sex ratios.

Among the species used to demonstrate a tendency for female biases is *Graptemys ouachitensis*, the Ouachita map turtle, with an average hatchling sex ratio of 1.9 : 1 in the field (female : male; Bull and Charnov, 1989). Mean field nest temperatures for *G. ouachitensis* fall between 24 and 30°C (Bull, 1985), although transitory nest temperatures as high as 37°C occurred during daily fluctuations (Bull and Vogt, 1979). These map turtles exhibit pattern I temperature-dependent sex determination (Ewert *et al.*, 1994), producing male offspring at cool temperatures and female offspring at warm temperatures. In the laboratory, incubation at constant temperatures below 28°C produces males, incubation above 30.5°C produces females and incubation at intermediate temperatures produces both sexes (Bull and Vogt, 1979; Bull *et al.*, 1982; Ewert and Nelson, 1991; M.A. Ewert, unpublished data).

Although variation in fitness due to thermal environment was, until recently, the only mechanism suggested as an explanation for naturally occurring primary sex-ratio bias in reptiles with environmental sex determination (Bull and Charnov, 1989), the theory has never been tested directly with data from the field or laboratory. We explored the effects of incubation environment on various traits related to fitness in Ouachita map turtles.

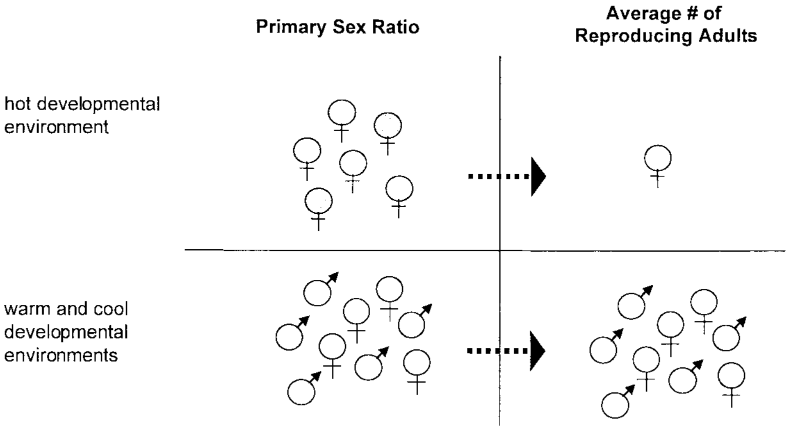

Fig. 1. Hypothetical effects of incubation temperature that would favour female-biased sex allocation in reptiles with pattern I temperature-dependent sex determination. Hot developmental environments produce only females and are also poor for fitness, such that only a small fraction of offspring produced there are fecund. The remaining environments are selected to produce a sex ratio close to 1 : 1, since the genetic contribution from the hot environment is minimal. The result is a biased primary sex ratio (2 : 1) favouring the hot environment sex (females), while the genetic contribution of each sex is functionally equal (1 : 1).

Specifically, we examined the effects of incubation temperature on survival, growth, performance and latency to move in juvenile *G. ouachitensis* for 9 months. Mark and recapture studies in other turtles with environmental sex determination suggest that size and locomotor performance affect fitness (Janzen *et al.*, 2000), while latency to move is positively correlated with survival (Janzen, 1995). Importantly, we incorporated a range of incubation temperatures comparable to the range of effective mean nest temperatures for map turtles in the wild (Bull, 1985). Our goal was to determine whether the effects of incubation temperature on fitness can explain female-biased sex ratios observed in map turtles. We found that male-producing temperatures resulted in lower fitness than female-producing temperatures, suggesting that environmental effects on fitness cannot explain the observed female bias.

METHODS AND MATERIALS

We collected eggs of *G. ouachitensis* between June and July 1999 along the West Fork of the White River, Daviess and Knox counties, Indiana. Eggs were obtained both through collection from newly laid nests and from gravid females. Gravid females were captured while nesting and induced to oviposit in the laboratory by injections of 0.7–0.8 ml of oxytocin (Ewert and Legler, 1978); they were then released. Eggs were placed in plastic boxes with moistened vermiculite (vermiculite : water, 1 : 1 by mass; *c*. 170 kPa) and were distributed among seven temperature-controlled incubators until hatching.

We randomly distributed a portion of eggs from 19 large clutches among seven incubation temperatures. Eggs that were clearly infertile were not used. The distribution from each clutch was roughly proportional to the number of eggs allocated to each treatment in the entire study. Eggs were placed at a range of constant incubation temperatures encompassing the natural range for *G. ouachitensis*. Constant incubation temperatures equivalent to variable field nest temperatures have been estimated for *G. ouachitensis* by comparing offspring sex ratios from wild nests with those from constant-temperature incubators (Bull, 1985). This comparison revealed that the range of ‘effective’ mean nest temperatures approximates a range of constant incubation temperatures from 24 to 31°C. In total, we placed 27 eggs at 33°C, 26 eggs at 31°C, 21 eggs at 29°C, 21 eggs at 27°C, 25 eggs at 25°C, 27 eggs at 24°C and 33 eggs at 22.5°C. More eggs were placed at the extreme temperatures to compensate for an expected increase in mortality during development at these temperatures.

After pipping, but before emerging from the shell, the turtles were placed in moist cups and maintained at 25°C. After the turtles had completely emerged from the eggshell, the shell was removed and enough water was added to completely cover the hatchling.

We ran performance trials on hatchlings at 2 weeks of age. They were weighed on the following day. At 30 days post-pip, each turtle was marked for identification by shell notching and placed in a 30 gallon plastic tub, measuring 86 × 58 × 20 cm, equipped with a filter and heater. Basking sites were added at 5 months after considerable mortality had occurred; mortality rates subsequently decreased. Water temperature was maintained at 25–28°C, while basking sites reached 30–34°C. Each tub housed approximately 30 turtles. The turtles were randomly distributed with respect to incubation temperature among the five tubs. Twice a week, we fed the turtles enough Trout Chow® pellets to ensure that there was excess remaining in each container, thus limiting any possible effects of competition for food.

Survival and growth

We measured survival as the proportion of individuals from each treatment alive at a given age. Survival was noted for two intervals: from oviposition to pipping and from pipping to 9 months (±7 days). Growth was recorded as the overall change in mass between 15 days post-pip and 9 months of age.

Performance

We chose righting response as a measure of performance because it is easily quantifiable and turtles are highly motivated to right themselves when placed upside down. At 14 ± 1 days post-pip, each turtle was placed upside down into one of nine open containers measuring 9 × 9 × 9 cm, aligned in a 3 × 3 formation on a plastic tray. Trials were videotaped on a Hi8 recorder while the experimenter was out of the room. The runs were checked every 10 min. Videotaping was stopped once all turtles had successfully righted. 'Time to right' is the total amount of time each turtle spent actively trying to right itself. This was scored as the time elapsed from when the turtle began pushing off the surface of the container until the turtle successfully righted itself, but minus the time the turtle was motionless once the righting attempt had begun. 'Latency to move' is the time elapsed from when the turtle was first placed in the container until it began attempting to right itself. Performance trials were run at ambient temperatures of approximately 23°C.

Statistical analyses

Statistical analyses were performed using SPSS® for Windows version 10.0. We determined the association of incubation temperature and hatchling mass with survival over the two intervals studied using logistic regressions. Analyses were conducted with all incubation temperatures, as well as with only those within the range of naturally occurring effective mean nest temperatures (i.e. 24–31°C). We determined the effect of incubation temperature on hatchling mass and growth using a one-way analysis of variance. Logistic regression analyses were used to examine the relationship between hatchling mass and survival over the two intervals studied. Effect of incubation temperature on righting time and latency time was tested by one-way analysis of variance after the data were transformed to produce homogeneous variances among the incubation treatments. We used Scheffé's *post-hoc* test to check for significant differences in hatchling growth, righting time and latency among incubation treatments. A logistic regression analysis was used to establish the relationship between righting time and survival to 9 months post-pip.

RESULTS

Of 180 eggs incubated, 120 produced live hatchlings. Considerable pre-pipping mortality occurred at 22.5°C (26 of 33 died) and 24°C (11 of 27 died). Clutch identity affected survival to pipping, as three clutches suffered mortality rates of 50% or more over all temperatures. Incubation temperature did not have a significant effect on survival to pipping for the entire study group. However, it was a good indicator of survival to pipping in the clutches with normal viability – that is, after the three high-mortality clutches were removed from the analysis (d.f. = 1, Wald statistic = 15.353, $P < 0.001$). Incubation temperature was also a good predictor of survival from pipping to 9 months (all clutches, d.f. = 1, Wald statistic = 21.672, $P < 0.001$). In each case, survival was lowest at the coolest incubation temperature. The effect of temperature on post-hatching survival remained significant when only incubation temperatures within the range of natural mean nest temperatures (24–31°C) were considered (Fig. 2; d.f. = 1, Wald statistic = 12.813, $P < 0.001$). Furthermore, the effect of survival appeared independent of any direct effects of gender, as cooler temperatures resulted in lower post-hatching survival within the range of temperatures that produces male offspring only (24–27°C; d.f. = 1, Wald statistic = 10.983, $P < 0.001$).

Post-hatching growth (to 9 months) was also affected by incubation temperature (Fig. 3; d.f. = 3, $F = 2.906$, $P < 0.05$). Growth was generally greater at warmer incubation temperatures. *Post-hoc* analysis among temperatures showed a marginally significant difference between turtles incubated at 25 and 31°C ($P = 0.054$). Within the natural range of

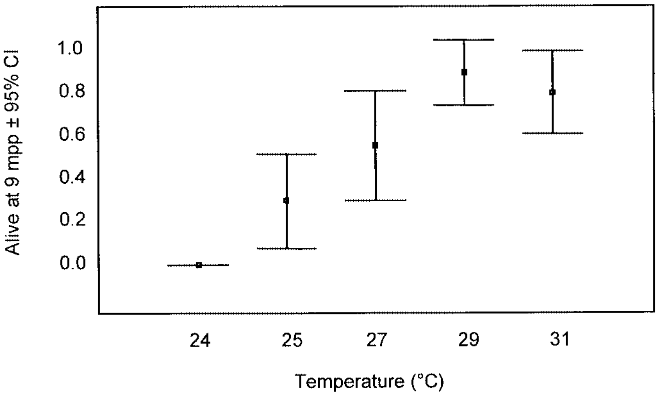

Fig. 2. Incubation temperature versus percent alive at 9 months. mpp = months post-pip.

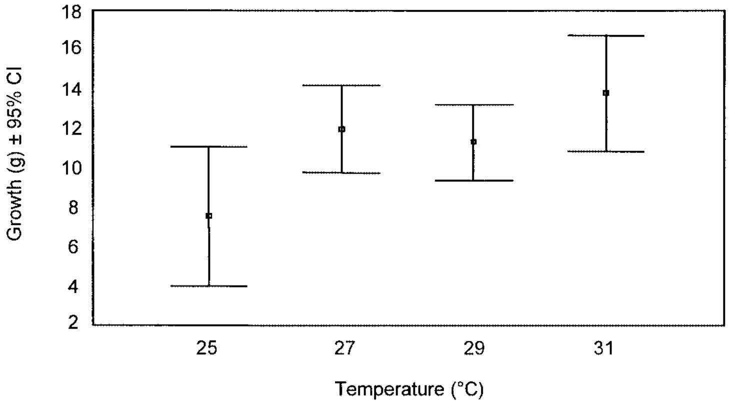

Fig. 3. Incubation temperature versus growth.

incubation temperatures (24–31°C), hatchling mass was not correlated with incubation temperature ($P = 0.677$) or with post-hatching survival to 9 months ($P = 0.301$).

Incubation temperature was a good predictor of a turtle's ability to right itself. Turtles incubated at cooler temperatures took longer to right themselves than turtles incubated at warmer temperatures (Fig. 4; d.f. = 4, $F = 22.911$, $P < 0.001$). *Post-hoc* analysis revealed significant differences in righting time between 24°C and all other temperatures ($P < 0.001$ for all four comparisons), as well as between 25 and 29°C ($P < 0.01$). Temperature also affected latency to move: turtles incubated at warmer temperatures waited longer before attempting to right themselves (Fig. 5; d.f. = 4, $F = 6.635$, $P < 0.001$). Latency was significantly different between 31 and 24°C ($P < 0.02$), 31 and 25°C ($P < 0.01$) and 31 and 27°C ($P < 0.005$).

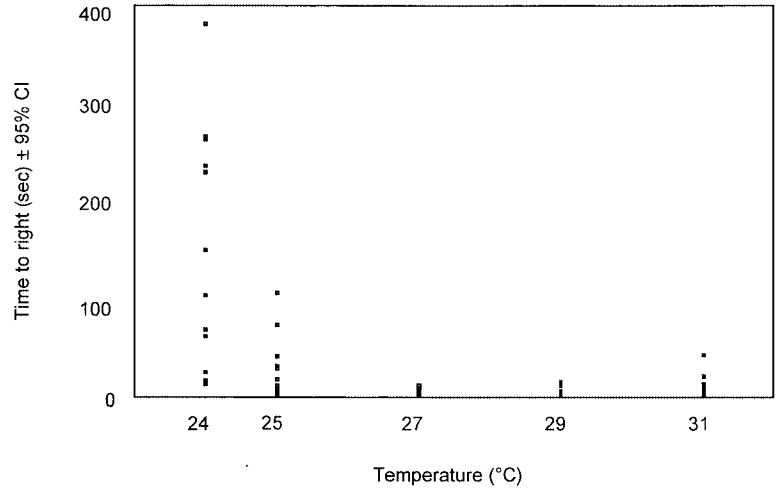

Fig. 4. Incubation temperature versus time to right.

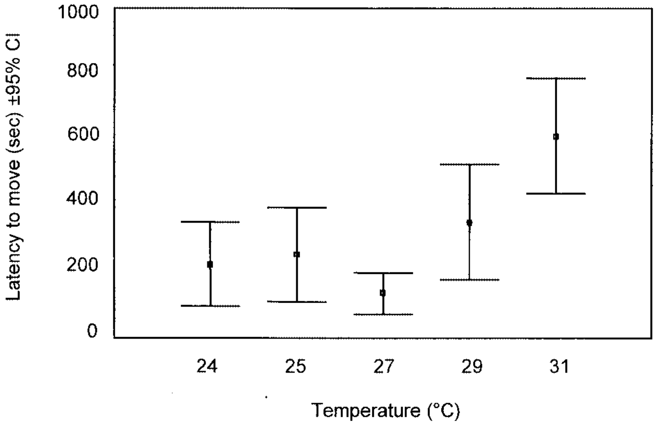

Fig. 5. Incubation temperature versus latency to move.

DISCUSSION

We assessed the effects of incubation temperature on fitness in *G. ouachitensis* to establish if differences in fitness due to environment could account for naturally observed sex-ratio biases. We found that male-producing temperatures yielded lower fitness in map turtles. Since theoretical models predict that the sex produced in the environments that yield lower fitness will be most abundant in the primary sex ratio, our results suggest that sex allocation in map turtles should be male-biased. The covariance between environment and fitness that we observed cannot be the cause of the observed skewed population sex ratios in map turtles, because our results predict a male bias, whereas field sex ratios observed for map turtles are strongly female-biased (reviewed in Bull and Charnov, 1988, 1989).

We observed the effect of incubation temperature on several phenotypic characters that probably contribute strongly to lifetime fitness. Our analyses demonstrated significant effects of incubation temperature on phenotype and, importantly, showed substantial differences between the ranges of temperatures equivalent to those that produce each sex in the wild. Specifically, we found that male-producing temperatures (cooler temperatures) resulted in offspring that grew slower, took longer to right themselves, had reduced latency to move and had lower survival to the age of 9 months. In addition, the trend of cooler temperatures producing poorer offspring also occurred among temperatures that produced only males, indicating that this variation was attributable to incubation temperature and not to gender.

Although we did not incorporate daily temperature fluctuation, it is unlikely that the strong pattern of male temperatures yielding lower fitness that we observed would disappear or be reversed in natural nests. Bull (1985) found that the effects in the laboratory of a constant incubation temperature on sex determination in *Graptemys* approximated the effects of fluctuating temperatures in the field sufficiently well to allow for replacing fluctuating temperatures with constant temperatures in laboratory experiments. In *Gopherus polyphemus*, a tortoise with environmental sex determination, there was no difference observed between the effects of fluctuating nest temperatures and equivalent constant incubation temperatures on hatchling performance and growth (Demuth, 2001). In *Apalone spinifera*, a turtle with genetic sex determination, no difference was found for burying speed between turtles incubated at fluctuating temperatures and their equivalent constant temperatures, although some differences were observed between certain fluctuating temperatures and their constant equivalents for some other phenotypic characters (Doody, 1999).

An additional factor that may affect the observed behaviour is the ambient temperature of the performance trials. O'Steen (1998) found that incubation temperature affected post-hatching thermal preference in juvenile snapping turtles, suggesting that thermal optima may vary with incubation temperature. Although the present study explored performance under only one set of environmental conditions (~23°C), results from a subsequent study in our laboratory support our current findings under different environmental conditions. *Graptemys ouachitensis* incubated at cooler temperatures took longer to right and show reduced latency regardless of ambient temperatures (30 *vs* 25°C; $P < 0.05$; S. Freedberg *et al.*, unpublished data).

If environmental effects on fitness do not predict the observed female-biased sex allocation in map turtles, then why are map turtle sex ratios female-biased? One part of the answer might be weather. The studies used to make the claim of biased sex ratios in map turtles relied on comprehensive hatchling sex ratios from 1980 (Vogt and Bull, 1984) and 1982 (Bull, 1985) in western Wisconsin. Mean July temperatures in both years were warmer than average, which may have contributed to the observed female bias. In 1980, when hatchling sex ratios were 3.2 : 1, mean July temperature at La Crosse, WI (about 10 miles from the study site) was 3.1°C higher than the long-term July average (NOAA weather records, 1980), the fifth warmest July recorded in La Crosse over the last 106 years. Mean July temperature at La Crosse in 1982, when the sex ratio was 1.2 : 1, was 1.1°C higher than average (NOAA weather records, 1982). July coincided with the thermosensitive period for sex determination for most turtles in these studies. The fact that map turtles produce females in the warmest environments suggests that the female bias inferred from these studies could result in whole or part from yearly variation in temperature and may not accurately represent equilibrium sex allocation.

Although the sex ratios observed for map turtles and some reptiles may be affected by yearly climate variation, the observations of 4 : 1 population sex ratios in map turtles are consistent with equilibrium female-biased sex allocation. Published population sex ratios for *G. ouachitensis* and the closely related *G. pseudogeographica* are approximately four females per male from both Wisconsin (Vogt, 1980) and South Dakota (Timken, 1968). In both studies, sampling techniques were reported as sexually unbiased. Yearly variation in ambient temperatures cannot account for a biased sex ratio when the adult population consists of turtles from several decades of nesting years. Although adult sex ratios do not necessarily represent primary sex ratios, there is no evidence of life-history differences between the sexes that would explain the strongly biased adult sex ratio. For differences in mortality to account for the sex ratio, the female life span would have to be more than four times greater than male life span. Vogt (1980) reported no evidence of differences in life span between male and female map turtles. Furthermore, differences in age at maturity between the sexes cannot explain the female bias, since male map turtles mature earlier than females. One other factor that may affect the observed sex ratios in Wisconsin is the presence of artificial dredge spoils in the nesting habitat, which might have resulted in a higher frequency of open, warm nesting environments (Bull and Charnov, 1988). It is not clear, however, that these spoils have altered enough nesting habitat to account for the 4 : 1 population-wide sex ratio. Thus, the heavily biased adult sex ratios suggest long-term female-biased sex allocation in *G. ouachitensis*.

The consistent finding of female-biased, primary and hatchling sex ratios in many studies of reptiles with environmental sex determination (see tables in Bull and Charnov, 1988; Ewert and Nelson, 1991; and Freedberg and Wade, 2001) indicates that female-biased sex allocation is a naturally occurring phenomenon in many reptiles with environmental sex determination. If other reptiles with the same pattern of sex determination exhibit a fitness response to incubation temperature similar to that of map turtles, then environmental effects on fitness cannot explain the biased sex ratios observed in these species. Importantly, cool incubation conditions have been found to compromise hatchling coordination in several diverse reptiles (Ewert, 1979; Burger, 1989; Ewert and Nelson, 1991; Janzen, 1993b; Bobyn and Brooks, 1994b; Spotila *et al.*, 1994; Ewert and Wilson, 1996; Harlow and Shine, 1999; Qualls and Andrews, 1999), indicating this pattern of environmental effects on fitness is conserved among reptiles.

Sex-determining pattern and sex ratios

Many reptiles with environmental sex determination produce predominantly or exclusively females, rather than males, at cool temperatures. The skewed sex ratios of these reptiles may be more congruous with variation in fitness associated with developmental environment. Some reptiles with environmental sex determination, including alligators and snapping turtles, produce males at intermediate incubation temperatures and predominantly females at both of the extremes (pattern II temperature-dependent sex determination; Ewert *et al.*, 1994). In these species, it is more likely that some of the naturally occurring female-producing temperatures are stressful to the developing embryos, thus resulting in decreased fitness and female-biased allocation.

Lance *et al.* (2000) note that primary sex ratios of alligators are decidedly female-biased, whereas adult sex ratios are equal or slightly male-biased. They suggest sex-specific mortality as a cause for the disparity. However, it is necessary to distinguish sex-specific mortality due to sex *per se* and mortality due to the different thermal environments that yield the two sexes. The differences between hatchling and adult sex ratios are consistent with either type of mortality. Specifically, some of the temperatures that produce female offspring may yield hatchlings of low fitness, causing females to have higher juvenile mortality rates, without any effects of gender on fitness *per se*. This interpretation would provide an explanation for the non-Fisherian primary sex ratios: the sex produced by the environment that is poorer for fitness is most abundant. In contrast, sex-specific mortality is not predicted to result in a similar primary sex-ratio bias (Appendix 1; Shaw and Mohler, 1953).

In snapping turtles, *Chelydra serpentina*, where a female-biased hatchling sex ratio has been reported (Schwarzkopf and Brooks, 1985), incubation temperature significantly affects post-hatching growth rate (Brooks *et al.*, 1991; McKnight and Gutzke, 1993; Bobyn and Brooks, 1994a,b; Rhen and Lang, 1995; O'Steen, 1998). Although all of these studies used a limited number of incubation treatments, combining their results reveals a strong trend associated with post-hatching growth and the range of temperatures known to produce each sex. Every incubation temperature that produced predominantly or exclusively males resulted in growth rates above the average for the particular study, while every temperature that produced predominantly or exclusively female offspring resulted in growth rates lower than average for the study (Table 1). Since hatchling size is known to be positively correlated with survival in the field for snapping turtles (Janzen, 1993a), these results strongly suggest that male-producing temperatures result in growth rates that increase fitness. This observation, in addition to the enhanced hatching success (Brooks *et al.*, 1991; Bobyn and Brooks, 1994a) and survival (Bobyn and Brooks, 1994a; Janzen, 1995) at male temperatures, implies that female-producing incubation temperatures yield lower fitness in snapping turtles and should consequently favour female-biased allocation. Thus the temperature effects on fitness found in these studies of a species with pattern II temperature-dependent sex determination are consistent with the observation of a female-biased hatchling sex ratio in snapping turtles, in contrast to what our results suggest for pattern I species.

It is unclear, however, whether the environmental effects on fitness in pattern II reptiles are of the magnitude necessary to produce evolutionarily stable, strongly skewed sex ratios. Theory predicts that large skews can occur only when the developmental environment exerts extreme effects on fitness (Bull, 1983). Since the primary sex ratio is proportional to the ratio of fitness resulting from male-producing temperatures to the fitness resulting from

Table 1. Relative growth rate from temperatures producing predominantly or exclusively one sex in snapping turtles

	Sex ratio (% female)	Relative growth rate
Female temperature		
21.1°C[b]	0.78	0.761
22°C[c]	0.81	0.953
22°C[a]	0.84 †	0.97
Male temperature		
24°C[f]	0	1.048
24.5°C[e]	0	1.12
25.3°C[b]	0.03	1.239
25.5°C[a]	0.18	1.145
25.6°C[c]	0	1.163
26.5°C[f]	0	1.068
27°C[d]	0 *	1.45
Female temperature		
28.6°C[c]	0.67	0.883
29°C[f]	0.84	0.884
29.3°C[a]	0.98	0.885
30.5°C[e]	1	0.8
31°C[d]	1*	0.857

[a] Bobyn and Brooks (1994a); [b] Bobyn and Brooks (1994b); [c] Brooks *et al.* (1991); [d] McKnight and Gutzke (1993); [e] O'Steen (1998); [f] Rhen and Lang (1995).

Note: Temperatures shown above are those temperatures found to produce a sex-ratio bias (greater than 60% of either sex). Temperatures producing more even sex ratios were not used. Sex ratios given are those found in the particular study. Relative growth rates are defined as the growth rate observed at that incubation temperature, divided by the average of the growth rates observed in the particular study.

* Sex determination was not performed in this study; sex determination was inferred from Yntema (1976).

† Due to fluctuating temperatures in the incubators, temperatures during sex determination were slightly different than the mean incubation temperatures listed.

female-producing temperatures (Bull and Charnov, 1989), a sex ratio of 2:1 (female:male) would require that female-producing temperatures confer half of the fitness of male-producing temperatures. The differences we found between males and females in survival, growth and performance in the laboratory suggest that appreciable differences in fitness should be expected under field conditions. However, it is not clear whether the difference would be as large as 50%.

Models for primary sex ratios

Until recently, the 'variable fitness' model examined in this study has been the only model proposed to explain biased primary sex ratios in reptiles. Freedberg and Wade (2001) recently predicted that skewed primary sex ratios can occur when a cultural trait is inherited asymmetrically (i.e. through one sex). In reptiles with environmental sex determination, this 'cultural transmission' model predicts that female biases will accrue when the nest-site location is culturally inherited from mother to daughter through homing to the natal site, a phenomenon termed nest-site philopatry. Specifically, the cultural model predicts that selection will favour female-producing nesting locations, since the nest site is limited in its transmission by the number of daughters produced there.

In contrast to the variable fitness model, the cultural transmission model is not dependent on environmental effects on lifetime fitness. This model relies instead on the occurrence of nest-site inheritance (reviewed in Bowen, 1995) and environmental sex determination, two phenomena strongly supported by data from some reptilian systems. Although our findings do not provide direct support for the cultural transmission model, their apparent incongruency with the environmental fitness effects model suggests that the cultural transmission model may be more consistent with known reptilian biology. The understanding of reptilian sex ratios should be aided by studies on environmental fitness effects and nest-site inheritance in a wider range of species, as well as studies of hatchling sex ratios across several years.

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APPENDIX

Sex allocation and fitness: environmental effects *vs* sex effects

Although the finding of differential mortality rates associated with sex in species with environmental sex determination can contribute to our understanding of primary sex ratios, it is imperative to distinguish environmental effects on fitness associated with sex from sex-specific fitness effects. Whereas the former, as we have discussed, will favour biased sex allocation, the latter scenario is predicted to result in even primary sex ratios. Shaw and Mohler (1953) demonstrated how sex-specific survival fails to affect the ESS sex ratio in their model for fitness of a sex ratio mutant, and Charnov (1982) discussed the role of sex-specific survival on sex allocation.

In the Shaw-Mohler model, K is defined as the number of grandchildren produced by the mutant female, S_m is male survival, C is clutch size, N is the total number of reproductive females in the population, r is the population sex ratio and r' is the sex ratio produced by the mutant female. Fitness through male function (W_m) is equal to:

$$W_m = 1/2\ (K)[(S_m Cr')/(S_m Cr' + NS_m Cr)] \tag{1}$$

Female fitness (W_f) is equal to:

$$W_f = 1/2\,(K)[\{S_f C(1-r')\}/\{S_f C(1-r') + NS_f C(1-r)\}] \quad (2)$$

where S_f is female survival. Total fitness (W_t) for the mutant female is then equal to:

$$W_t = 1/2\,(K)[\{S_f C(1-r')\}/\{NS_f C(1-r)\} + \{S_m Cr'\}/\{NS_m Cr\}] \quad (3)$$

After removing the proportionality factor, 1/2 (K/N), this reduces to:

$$W_t \propto [(1-r')/(1-r) + r'/r] \quad (4)$$

The critical element to note here is that the sex-specific survival rates, S_m and S_f, drop out of the final equation and do not affect the fitness of the sex-ratio mutant.

Unlike sex differences in fitness, environmental effects that produce differences in the average fitness of each sex are predicted to result in biased sex allocation. This process is outlined in Fig. 1 and further demonstrated by the equilibrium primary sex ratio under environmental sex determination, as shown by Bull and Charnov (1989). In their model, τ is defined as the environment that produces a 1 : 1 sex ratio, r is the proportion male at conception, $W_m(\tau)$ is the relative fertility of a male exposed to (τ), $W_f(\tau)$ is the relative fertility of a female exposed to (τ), and W_m and W_f are the average fertilities of male and females in the population, respectively. The primary sex ratio (proportion male) at equilibrium is ultimately:

$$r/(1-r) = [W_m(\tau)/W_m]/[W_f(\tau)/W_f] \quad (5)$$

It is clear that fitness of each sex does affect sex allocation, such that differences in survival between the sexes resulting from developmental environment will alter the primary sex ratio.