

Experimental Test of the Effects of Fluctuating Incubation Temperatures on Hatchling Phenotype

HEATHER L. LES, RYAN T. PAITZ, AND RACHEL M. BOWDEN*

Behavior, Ecology, Evolution, and Systematics Section,

Department of Biological Sciences, Illinois State University, Normal, Illinois

ABSTRACT In the painted turtle (*Chrysemys picta*) and red-eared slider turtle (*Trachemys scripta*), the temperature that eggs are exposed to during incubation determines the sex of the developing embryo. Constant temperature incubation experiments have shown that for each of these species there is a pivotal temperature that produces a 1:1 sex ratio; higher temperatures bias sex ratios toward females, and lower temperatures toward males. Few studies have examined how fluctuating temperatures, as would be experienced in natural nests, affect hatchling phenotype. Models predict that under fluctuating temperatures sex determination depends on the proportion of development that occurs above or below the pivotal temperature. We tested the effect of fluctuating versus constant temperature incubation regimes on sex ratios and other hatchling traits for both painted and red-eared slider turtles. Eggs were divided into two treatments with half of the eggs from each species incubated at a constant intermediate temperature, 28.5°C, and half incubated under temperatures that fluctuated 3°C above and below 28.5°C. We converted the fluctuating temperature data into a constant temperature equivalent (CTE) so that we could directly compare constant and fluctuating incubation regimes. The CTE for the fluctuating regime for both species was higher than the constant temperature, which would predict an increase in the production of females. The fluctuating regime did produce a higher proportion of females, but also resulted in increased developmental time and increased hatchling mass, indicating that fluctuating temperatures produce complex effects on hatchling phenotype. *J. Exp. Zool.* 307A:274–280, 2007. © 2007 Wiley-Liss, Inc.

How to cite this article: Les HL, Paitz RT, Bowden RM. 2007. Experimental test of the effects of fluctuating incubation temperatures on hatchling phenotype. *J. Exp. Zool.* 307A:274–280.

Incubation temperatures experienced by reptilian eggs influence many aspects of a hatchling's phenotype. Traits such as size, morphology, locomotor performance, behavior, and sex can all be affected by temperature (reviewed in Deeming, 2004). Temperature effects during development are assumed to have fitness consequences, but direct empirical evidence to support this assumption has been difficult to come by because the maturation process in many reptiles is slow and so following hatchlings to maturity is often impractical. Laboratory studies have been employed to investigate the effects of incubation temperature on fitness. These studies have primarily utilized constant temperatures and fitness surrogates to estimate the effects of incubation temperature on fitness and report that, in general, increasing incubation temperature results in shorter incubation times and increased locomotor performance

in turtles (Janzen, '93; Du and Ji, 2003), whereas elevating temperatures resulted in decreased performance in lizards (Qualls and Andrews, '99; Braña and Ji, 2000). Studies have also shown that fluctuating incubation temperatures can influence hatchling phenotypes differently than constant temperatures (Shine et al., '97; Ashmore and Janzen, 2003; Mullins and Janzen, 2006; but see Demuth, 2001), but the phenotypic outcomes vary depending upon experimental conditions including the magnitude of fluctuations (Georges et al., '94).

Grant sponsor: National Science Foundation; Grant number: IBN0212935.

*Correspondence to: R.M. Bowden, Behavior, Ecology, Evolution, and Systematics Section, Department of Biological Sciences, Illinois State University, Normal, IL 61790. E-mail: rmbowde@ilstu.edu

Received 6 November 2006; Revised 11 January 2007; Accepted 16 January 2007

Published online 20 March 2007 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jez.374.

The relative paucity of studies focusing on the effects of temperature makes it difficult to evaluate the generality of the observed fitness outcomes under either constant or fluctuating regimes, but fluctuating regimes should better mimic the conditions experienced in natural nests, and thus provide a more realistic method for examining how temperature influences both hatchling phenotypes and fitness.

The fact that sex can be affected by temperature has intrigued biologists and spawned numerous theories to explain why a trait with such clear fitness consequences should be left to the vagaries of the environment. Temperature-dependent sex determination (TSD) is found in all crocodylians, most species of turtles, some species of lizards, and the tuatara. In reptiles with TSD, sex is determined after fertilization and reproductive maturity may not occur for several years beyond the point of sex determination. In turtles with TSD, either higher temperatures produce females, whereas lower temperatures produce males (TSD pattern I), or high and low temperatures produce females with males produced at intermediate temperatures (TSD pattern II; Ewert and Nelson, '91; Ewert et al., '94). This inherent delay between fertilization, sex determination, and reproductive maturity in reptiles with TSD has complicated studies aimed at coupling the effects of temperature on sex determination and subsequent fitness (e.g., Roosenburg and Kelly, '96). Nonetheless, a rich body of theory has been developed to help explain the potential adaptive advantage and persistence of TSD (Charnov and Bull, '89; Ewert and Nelson, '91; Shine, '99). The most popular theoretical foundation used to explain the adaptive significance of TSD is the Charnov-Bull model, which relies upon sex-specific differential fitness to explain the presence of TSD in reptiles. According to this model, organisms inhabit a heterogeneous environment where male fitness will be higher in some incubation patches and female fitness will be higher in other incubation patches. To date, tests of this model have failed to provide much support for sex-specific differential fitness in reptiles, however most studies have used constant incubation regimes; conditions that are not representative of those experienced in natural nests. Constant temperature studies have nonetheless demonstrated that temperature does produce a relatively predictable effect on sex determination, with each species (and sometimes populations within a species) having a pivotal temperature (temperature that produces a 50:50

sex ratio; Yntema and Mrosovsky, '82) and an optimal developmental temperature range (Ewert and Nelson, '91; de Souza and Vogt, '94; Valenzuela, 2001; Willingham, 2005).

Georges ('89) proposed a model to predict how temperatures fluctuating equally above and below a stationary mean would affect sex determination in turtles with TSD pattern I. The model assumes a linear relationship between the rate of embryonic development and incubation temperature and predicts that females will be produced if greater than half of embryonic development takes place at temperatures above the pivotal temperature, whereas males will be produced if greater than half of embryonic development occurs below the pivotal temperature. This prediction arises from the fact that more embryonic development takes place when temperatures exceed the pivotal temperature, suggesting that sex determination hinges upon the proportion of development spent above the pivotal temperature, not the proportion of time spent above it (Bull and Vogt, '81; Georges et al., '94). The model also allows for the determination of a constant temperature equivalent (CTE) for fluctuating incubation regimes, which is the temperature at which half of development occurs above it and half occurs below it. The CTE can be used to compare fluctuating temperature regimes with constant temperature regimes with the expectation that constant temperature incubations at the CTE of a fluctuating regime will produce similar sex ratios as the fluctuating regime (Georges et al., '94). Reanalysis of several previous studies provided empirical support for this model as a valuable tool for predicting sex ratios from fluctuating incubation regimes, provided that the fluctuations do not reach extreme developmental temperatures (Georges et al., 2005). When incubation temperature does reach an extreme, developmental rate is retarded and thus is non-linear.

Temperature fluctuations are especially common in the shallow nests of many smaller freshwater turtles (Packard et al., '85; Georges, '92; Demuth, 2001). Understanding how fluctuating incubation temperatures influence hatchling phenotypes is critical to understanding how natural incubation conditions affect hatchling fitness. We tested the effects of fluctuating versus constant incubation temperatures on developmental parameters including sex determination, incubation length, and hatchling mass in two species of freshwater turtles (*Chrysemys picta* and *Trachemys scripta*). We chose a constant incuba-

tion temperature (28.5°C) that was well within the viable temperature range for both species. Based upon the model of Georges ('89) we hypothesized that our fluctuating regime would shift sex ratios by increasing the proportion of females, but would not affect either incubation length or hatchling mass relative to the constant temperature regime.

METHODS

Egg collection and incubation

Eggs were obtained from 13 painted turtles (*C. picta*) and 12 red-eared slider turtles (*T. scripta*) from Banner Marsh State Fish and Wildlife Area in Central Illinois during May and June of 2005. Gravid females of both species were caught in traps or on nesting forays and brought back to the lab to induce oviposition via oxytocin injection (see Etchberger et al., '92). One *T. scripta* female was captured post-laying and her nest excavated to collect recently laid eggs. After oviposition was complete, two eggs from each clutch were subsequently frozen to be used in a different study, leaving a total of 55 *C. picta* eggs and 107 *T. scripta* eggs. All eggs were weighed to the nearest 0.01 g and marked as to clutch and individual, were randomized with respect to clutch, and then placed into incubation boxes containing moistened vermiculite (~150 kPa). For each species, four boxes were used for each of the two experimental incubation regimes resulting in eight boxes being placed into each of the two programmable incubators (Memmert GmbH+Co.KG, Schwabach, Germany). No longer than 24 h passed between time of oviposition and the placement of eggs into the incubators. Each incubator had an internal chamber volume of 1.85 ft³, contained two shelves, and was equipped with Celsius 2000 software (Memmert GmbH+Co.KG, Schwabach, Germany), which allowed both execution of temperature programs and simultaneous recording of chamber temperature. One incubator ran at a constant temperature throughout development while the other incubator ran a program of continuous sinusoidal fluctuations. Individual box temperature was not monitored, but one temperature data logger was placed centrally in each incubator to monitor chamber temperature once every 30 min (iButton, Dallas-Maxim, Dallas, TX).

Eggs were arranged linearly and partially buried in the vermiculite for incubation either at a constant intermediate temperature (28.5°C) or at a temperature that fluctuated $\pm 3^\circ\text{C}$ around

28.5°C. The fluctuating temperature regime completed a cycle once every 24 h resulting in a mean temperature of 28.5°C (Fig. 1). Because of their smaller size, *C. picta* clutches were placed wholly into one of the two incubation regimes, resulting in 28 eggs in the constant incubation treatment (6–10 eggs/box) and 27 eggs in the fluctuating incubation treatment (6–7 eggs/box). Each *T. scripta* clutch was divided approximately into half, resulting in 55 eggs in the constant incubation treatment (12–15 eggs/box), and 52 eggs in the fluctuating incubation treatment (12–14 eggs/box). *C. picta* and *T. scripta* eggs were maintained in separate closed boxes within the two incubators. Water evaporation was checked every 5 days; water was added as necessary to maintain hydric conditions. Boxes were also rotated within each incubator every 5 days to minimize the effects of any inconsistencies in temperature throughout the incubator. As hatching approached, but after the temperature sensitive period was complete, all boxes were checked daily for pipping (first breach of the eggshell). Upon hatch, animals were removed from their respective boxes and maintained individually. We confirmed that the incubators ran as programmed by comparing the profiles generated by the incubator software and the thermochrons.

Measurements and sex determination

Incubation length was characterized as the time between oviposition and pipping. All hatchlings were weighed to the nearest 0.01 g and plastron length was measured to the nearest 0.01 mm at 10 days post-pip. All hatchlings were kept in

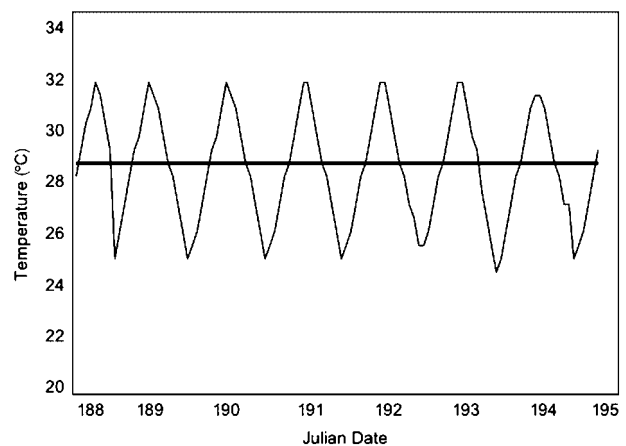


Fig. 1. Representative incubator temperature profiles for a 1-week period in July during the thermosensitive period. The mean of both the constant and fluctuating temperature regimes is 28.5°C.

incubators held at 26°C for 60 days post-hatch to ensure complete gonadal differentiation. After this time, sex was determined by macroscopic examination of gonads and Müllerian ducts (Bowden et al., 2000).

Data analyses

The mean values of each box of eggs were used as the experimental unit to test the effects of temperature treatment on hatchling measures. Differences in hatchling mass, plastron length, developmental time, and sex ratios were analyzed using ANCOVAs with egg mass as the covariate. Sex ratios were arcsine transformed before analysis while no other measures required transformation to meet the assumptions of the respective analyses. To estimate the effect of clutch on hatchling measures, we used a mixed model ANCOVA with temperature as a fixed factor, incubator box nested within treatment, clutch as a random factor, and with egg mass as a covariate. For all ANCOVAs, when initial egg mass was not significant, we removed the covariate from the model and reanalyzed the data using an ANOVA. To further investigate the relationship between incubation length and hatchling mass we ran a multiple linear regression using incubation length and egg mass as predictors of hatchling mass. Test statistics were generated using SAS, v. 9.1 (SAS Institute Cary, North Carolina). Developmental zeros (T_0) for the CTE calculations were determined by regressing the inverse of incubation time against temperature (Georges et al., 1994). Since our study tested only a single constant temperature, data from other studies were used for this estimation (*C. picta*: Gutzke et al., '87 and *T. scripta*: Paitz, personal communication), resulting in a T_0 of 14.0°C for *C. picta* and 16.0°C for *T. scripta*.

RESULTS

Initial egg mass did not differ between the constant and fluctuating incubation treatments (*C. picta*: $F_{1,53} = 0.22$, $P = 0.64$; *T. scripta*: $F_{1,105} = 0.72$, $P = 0.40$; Table 1). The CTEs for the fluctuating temperature regimes were determined to be 29.10°C for *C. picta* and 29.19°C for *T. scripta*, both higher than the constant incubation temperature. The fluctuating temperature regime resulted in longer incubation periods for both *C. picta* (ANOVA: $F_{1,7} = 36.65$, $P < 0.0009$) and *T. scripta* (ANOVA: $F_{1,7} = 12.84$, $P = 0.012$) relative to the constant regime (Table 1).

Hatchlings from eggs incubated under fluctuating temperatures were heavier than those incubated at constant temperatures; this pattern was not significant for *C. picta* (ANCOVA: $F_{2,7} = 5.29$, $P = 0.07$), but was for *T. scripta* (ANOVA: $F_{1,7} = 11.05$, $P = 0.016$). For both species, egg mass and incubation length were significant predictors of hatchling mass (multiple linear regression: *C. picta*: egg mass, $P < 0.0001$; incubation length, $P < 0.0001$; *T. scripta*: egg mass, $P < 0.0001$; incubation length, $P = 0.0321$; Fig. 2). Plastron length did not differ significantly between fluctuating and constant temperature regimes for either species (*C. picta*: $F_{1,7} = 0.001$, $P = 0.97$; *T. scripta*: $F_{1,7} = 0.86$, $P = 0.39$; Table 1). Clutch explained a significant amount of the observed variation in incubation period, hatchling mass, and plastron length for *T. scripta* ($P < 0.05$ in all three cases). The effect of clutch could not be tested in *C. picta* because their smaller clutch size resulted in entire clutches being placed into either constant or fluctuating conditions. For both species, the fluctuating temperature regime produced a significantly higher proportion of females compared with the constant temperature regime (*C. picta*: $F_{1,7} = 57.92$, $P = 0.0003$; *T. scripta*: $F_{1,7} = 9.41$, $P = 0.022$; Fig. 3).

DISCUSSION

Our study supports the idea that fluctuating incubation temperatures influence hatchling phenotypes differently than constant incubation temperatures. The general trend in constant temperature experiments is for higher temperatures to induce a shorter incubation period and smaller hatchlings (Booth, 2006). In species with pattern I TSD, higher temperatures also produce female biased sex ratios. In this study fluctuating temperatures shifted the sex ratio toward a higher proportion of females in both species as would be predicted from the higher calculated CTEs (Georges et al., '94). We also found that both incubation length and hatchling mass increased; outcomes typically associated with lower incubation temperatures. Although only two incubators were used in this experiment, the fact that some hatchling measures responded to fluctuating temperature in a manner consistent with warmer incubation temperatures while other measures responded in a manner consistent with cooler temperatures indicates that these effects are likely not just the result of incubator effects and can be attributed to temperature fluctuations. These

TABLE 1. Phenotypic parameter averages by species and incubation treatment

Treatment	Egg mass (g)	Hatchling mass (g)	Days of development	Plastron length (mm)
<i>Chrysemys picta</i>				
Constant	6.97	4.83	56	26.22
Fluctuating	7.06	5.26	58	26.36
<i>Trachemys scripta</i>				
Constant	12.06	8.60	61	31.59
Fluctuating	12.24	8.82	62	31.73

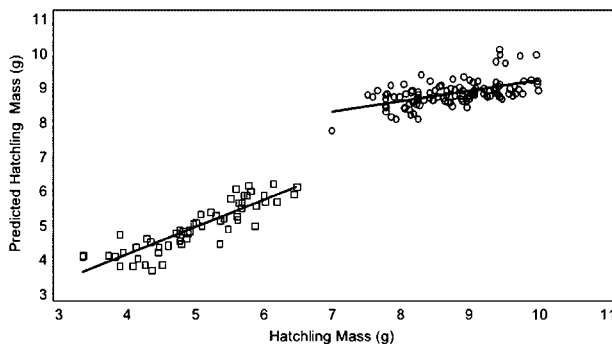


Fig. 2. Relationship between predicted and observed hatchling mass for *C. picta* (squares; $r_{adj} = 0.88$) and *T. scripta* (circles; $r_{adj} = 0.54$).

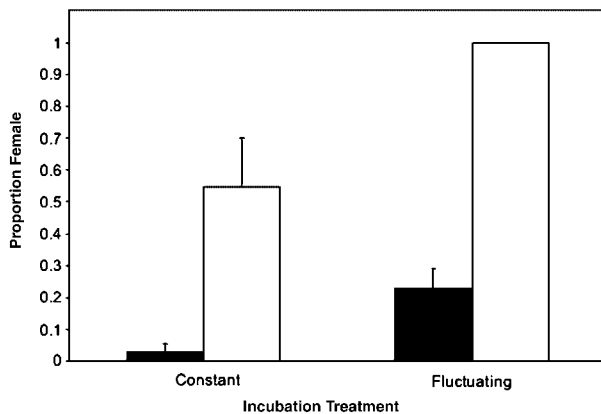


Fig. 3. Mean sex ratios (± 1 SEM) from eggs incubated at either a constant or fluctuating temperature regime. *T. scripta* sex ratios are in black bars and those for *C. picta* are in open bars. Under fluctuating conditions, *C. picta* produced all female hatchlings so there are no error bars associated with this group.

results suggest that variation in temperature during incubation has the potential to differentially impact a variety of fitness-related

parameters compared with constant temperature regimes.

Understanding how incubation conditions affect fitness is critical to testing theories regarding the evolution of TSD. To date, little empirical evidence exists to explain the persistence of TSD in reptiles, and this may be the result of the widespread use of constant temperature incubation regimes that do not mimic natural nests, with the possible exception of species that dig deep nests such as sea turtles (Booth, 2006). The extent to which fluctuating temperatures affect hatchling phenotypes is still unclear because of the limited number of studies addressing this issue. Those studies that have utilized fluctuating regimes have demonstrated that: (1) fluctuating temperatures allow eggs to survive short bouts of incubation at temperatures that would be lethal under constant conditions, and (2) fluctuating temperatures produce a greater proportion of females compared with constant temperature incubations conducted at the mean of the fluctuating regime in species with type I TSD (Bull, '85; de Souza and Vogt, '94; Demuth, 2001; this study). This latter effect is attributed to a greater proportion of development occurring at temperatures above the mean versus below the mean (Georges, '89). If fluctuations reach extreme temperatures during incubation, curvilinearity models predict a retardation of developmental rate that, in turn, would increase the time of development at high temperatures and decrease it at low temperatures (Georges et al., 2005). Longer developmental times are often associated with the production of larger and heavier hatchlings as a greater proportion of yolk is converted into tissue (reviewed in Deeming, 2004). Here, we show that fluctuating temperatures are associated with an increase in developmental time and hatchling mass compared with constant temperatures indicating that temperature fluctuations may play an important role in the evolution of TSD. Previous studies have found that incubation length is positively associated with egg mass (Birchard and Marcellini, '96; Deeming et al., 2006), and it is possible that our observed increase in incubation length could be attributed to variation in egg mass rather than to temperature fluctuations. We found that in general, larger eggs did take longer to develop, but that for a given egg mass longer developmental times are associated with significantly larger hatchlings (Fig. 2). The minimal increase in developmental time observed in this study may, on its own, be of limited biological significance as the hatchlings

of both species overwinter in their natal nests at this latitude (Tucker, 2000). However, if that increase in developmental time is related to the production of a more massive hatchling, as we report herein, then increasing incubation time may indeed be of biological importance and previous studies have demonstrated positive effects of hatchling mass on fitness. Whether or not greater temperature fluctuations may amplify this pattern of increasing incubation length and hatchling mass to the extent that hatchling fitness is affected is currently not known.

Elevated temperatures in natural nests are often associated with increasing variances, and the increased variance in temperature results in a greater likelihood of eggs experiencing temperature extremes that could increase developmental time and hatchling mass (de Souza and Vogt, '94; Shine and Harlow, '96). In the case of type I TSD, this would lead to an increased production of female hatchlings which are larger than those from constant temperature incubations, and this increase in hatchling mass may have important fitness consequences at several early life history stages. For example, previous research has demonstrated that hatchlings with greater lipid stores are better able to survive the freezing conditions commonly encountered by species that overwinter in their natal nests (Costanzo et al., 2000), and larger hatchlings have been shown to have increased survival during post-emergent migration (Janzen, '93; Tucker, 2000; Paitz et al., 2007). Thus the effect of fluctuating temperatures on hatchling phenotypes may contribute to differential fitness of males and females and could ultimately underlie why most constant temperature incubation studies have failed to detect any fitness differences.

The CTE model proposed by Georges ('89) was developed to predict sex ratio under fluctuating temperatures and here we report further support for this model in two species of Emydid turtles. That we observed variation in trait response to the fluctuating incubation regimes such that the shift in sex ratio was suggestive of an overall warmer incubation environment and the shift in incubation length and hatchling mass of an overall cooler incubation environment indicates that the CTE model is not applicable to all temperature sensitive traits. Nonetheless, it does appear to be a valuable tool for predicting sex ratios. The ability to translate fluctuating temperatures into a CTE has made it possible to determine any divergence of developmental parameters between constant

and fluctuating incubation regimes. By comparing our two regimes, we conclude that fluctuating incubation conditions produce complex effects on hatchling phenotype, but those effects appear to result in outcomes that would enhance offspring fitness and may promote the evolutionary maintenance of TSD in reptiles. Future studies should be conducted with a wider variety of species and with temperatures fluctuating about a wider range in an effort to better mimic natural nest conditions in shallow nesting species.

ACKNOWLEDGMENTS

We would like to thank the Illinois Department of Natural Resources for granting access to Banner Marsh and Arthur Georges for providing valuable comments on an earlier version of this manuscript. Animals were collected under IDNR permit NH05.2084 and research conducted following the Illinois State University IACUC guidelines.

LITERATURE CITED

- Ashmore GM, Janzen FJ. 2003. Phenotypic variation in smooth softshell turtles (*Apalone mutica*) from eggs incubated in constant versus fluctuating temperatures. *Oecologia* 134:182–188.
- Birchard GF, Marcellini D. 1996. Incubation time in reptilian eggs. *J Zool* 240:621–635.
- Booth DT. 2006. Influence of incubation temperature on hatchling phenotype in reptiles. *Func Ecol* 79:274–281.
- Bowden RM, Ewert MA, Nelson CE. 2000. Environmental sex determination in a reptile varies seasonally and with yolk hormones. *Proc R Soc Lond B* 267:1745–1749.
- Braña F, Ji X. 2000. Influence of incubation temperature on morphology, locomotor performance, and early growth of hatchling wall lizards (*Podarcis muralis*). *J Exp Zool* 286:422–433.
- Bull JJ. 1985. Sex ratio and nest temperature in turtles: comparing field and laboratory data. *Ecology* 66:1115–1122.
- Bull JJ, Vogt RC. 1981. Temperature-sensitive periods of sex determination in Emydid turtles. *J Exp Zool* 218:435–440.
- Charnov EL, Bull JJ. 1989. The primary sex ratio under environmental sex determination. *J Therm Biol* 139:431–436.
- Costanzo JP, Litzgus JD, Iverson JB, Lee RE Jr. 2000. Seasonal changes in physiology and development of cold hardiness in the hatchling painted turtle *Chrysemys picta*. *J Exp Biol* 203:3459–3470.
- de Souza RR, Vogt RC. 1994. Incubation temperature influences sex and hatchling size in the neotropical turtle *Podocnemis unifilis*. *J Herp* 28:453–464.
- Deeming DC. 2004. Reptilian incubation: environment, evolution and behaviour. Nottingham: Nottingham University Press. 349p.
- Deeming DC, Birchard GF, Crafer R, Eady PE. 2006. Egg mass and incubation period allometry in birds and reptiles: effects of phylogeny. *J Zool* 270:209–218.

- Demuth JP. 2001. The effects of constant and fluctuating incubation temperatures on sex determination, growth, and performance in the tortoise *Gopherus polyphemus*. *Can J Zool* 79:1609–1620.
- Du W-G, Ji X. 2003. The effects of incubation thermal environments on size, locomotor performance and early growth of hatchling soft-shelled turtles, *Pelodiscus sinensis*. *J Therm Biol* 28:279–286.
- Etchberger C, Ewert MA, Raper BA, Nelson CE. 1992. Do low incubation temperatures yield females in painted turtles? *Can J Zool* 70:391–394.
- Ewert MA, Nelson CE. 1991. Sex determination in turtles: diverse patterns and some possible adaptive values. *Copeia* 1991:50–69.
- Ewert MA, Jackson DR, Nelson CE. 1994. Patterns of temperature-dependent sex determination in turtles. *J Exp Zool* 270:3–15.
- Georges A. 1989. Female turtles from hot nests: is it duration of incubation or proportion of development at high temperatures that matters? *Oecologia* 81:323–328.
- Georges A. 1992. Thermal characteristics and sex determination in field nests of the pig-nosed turtle *Carettochelys insculpta* (Chelonia: Carettochelydidae), from northern Australia. *Aust J Zool* 40:511–521.
- Georges A, Limpus C, Stoutjesdijk R. 1994. Hatchling sex in the marine turtle *Caretta caretta* is determined by proportion of development at a temperature, not daily duration of exposure. *J Exp Zool* 270:432–444.
- Georges A, Beggs K, Young JE, Doody SJ. 2005. Modelling development of reptile embryos under fluctuating temperature regimes. *Physiol Biochem Zool* 78:18–30.
- Gutzke WHN, Packard GC, Packard MJ, Boardman TJ. 1987. Influence of the hydric and thermal environments on eggs and hatchlings of painted turtles (*Chrysemys picta*). *Herpetologica* 43:393–404.
- Janzen FJ. 1993. An experimental analysis of natural selection on body size of hatchling turtles. *Ecology* 74:332–341.
- Mullins MA, Janzen FJ. 2006. Phenotypic effects of thermal means and variances on smooth softshell turtle (*Apalone mutica*) embryos and hatchlings. *Herpetologica* 62:27–36.
- Packard GC, Packard MJ, Gutzke WHN. 1985. Influence of hydration on the environment on eggs and embryos of the terrestrial turtles *Terrapene ornata*. *Physiol Zool* 58:564–575.
- Paitz RT, Harms HK, Bowden RM, Janzen FJ. 2007. Experience pays: offspring survival increases with female age. *Biol Lett* 3:44–46.
- Qualls CP, Andrews RM. 1999. Cold climates and the evolution of viviparity in reptiles: cold incubation temperatures produce poor-quality offspring in the lizard, *Sceloporus virgatus*. *Biol J Linn Soc* 67:353–376.
- Roosenburg WM, Kelly KC. 1996. The effect of egg size and incubation temperature on growth in the turtle, *Malaclemys terrapin*. *J Herp* 30:198–204.
- Shine R. 1999. Why is sex determined by nest temperature in many reptiles? *TREE* 24:186–189.
- Shine R, Harlow PS. 1996. Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology* 77:1808–1810.
- Shine R, Elphick MJ, Harlow PS. 1997. The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology* 78:2559–2568.
- Tucker JK. 2000. Body size and migration of hatchling turtles: inter- and intraspecific comparisons. *J Herp* 34:541–546.
- Valenzuela N. 2001. Constant, shift, and natural temperature effects on sex determination in *Podocnemis expansa* turtles. *Ecology* 82:3010–3024.
- Willingham E. 2005. Different incubation temperatures result in differences in mass in female red-eared slider turtle hatchlings. *J Therm Biol* 30:61–64.
- Yntema CL, Mrosovsky N. 1982. Critical periods and pivotal temperatures for sexual differentiation in loggerhead sea turtles. *Can J Zool* 60:1012–1016.