

How frequent and important is behavioral thermoregulation by embryonic reptiles?

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Abstract

The debate about behavioral thermoregulation inside reptile eggs centers on the frequency (and hence, biological significance) of the phenomenon, not about its validity. Both sides of the debate agree that large eggs in shallow nests laid in sun-exposed soil will experience clines in mean temperature and (especially) diel thermal variance; that embryos in the middle phase of development have the ability to reposition themselves, and room to do so; and that small changes in developmental temperatures can influence offspring fitness. Equally, all protagonists agree that thermal clines will be too low in some other kinds of nests, and that embryonic repositioning is impossible very early and very late in development. Based on an array of other fitness-enhancing behaviors exhibited by tetrapod embryos, and general principles for recognizing adaptation, we conclude that behavioral thermoregulation inside the egg likely is adaptive in some but not all reptile species. We identify productive directions for empirical research to resolve points of contention.

KEYWORDS

embryo, fluctuating temperatures, heat-seeking, nest environment, thermoregulatory behavior

1 | INTRODUCTION

Behavioral thermoregulation is central to the life of reptiles, but until recently was presumed to occur only during post-hatching life. That assumption came under strong challenge from our experimental studies, which demonstrated that embryos of some (but not all) of the species studied (including turtles, squamates, and alligators) shift within the egg in response to thermal gradients. Specifically, embryos moved toward warmer regions, but away from lethally hot regions (Du, Zhao, Chen, & Shine, 2011; Li, Zhao, Zhou, Hu, & Du, 2014; Zhao, Li, Shine, & Du, 2013, 2014). Based on those data, we suggested that behavioral thermoregulation in reptiles can begin prior to hatching.

Our conclusion was contested by Telemeco et al. (2016) and Cordero, Telemeco, and Gangloff (2017), who argued instead that thermal heterogeneity within eggs, and the physical ability of embryos to reposition themselves, are too limited to allow scope for behavioral thermoregulation by embryos. We welcome the debate, and below we identify points of agreement and disagreement between the two sides of the argument, and address specific criticisms of our hypothesis.

1.1 | Clarifying points of disagreement

Unlike many debates in science, the present one involves broad agreement between the two “opposing” sides. The central issue is the *level of generality* of thermoregulation by embryos. We believe

that proponents (like ourselves) and opponents (like Telemeco et al., 2016 and Cordero et al., 2017) would agree to all of the following statements:

1. Under some circumstances (notably, within large eggs in shallow nests in sun-exposed locations), different places within the same egg can vary in mean temperature and especially, in thermal variation during the diel cycle.
2. Under other circumstances (small eggs, deep nests, shaded sites), there is minimal to zero thermal variation within a single egg.
3. During the middle part of development, an embryo is able to move within its egg (i.e., it has room to do so, and the necessary musculature).
4. Early and late in development, embryonic movement may be precluded by a lack of musculature (early development) or a lack of room to move (late development).
5. Buffering by the eggshell, and heat flow within an egg, reduce thermal differentials inside an egg below the levels measured at the external surface of that egg. That factor will reduce embryonic opportunities for behavioral thermoregulation, even in a nest where temperatures at the external surfaces of an egg exhibit significant spatial variation.
6. Even if the range of mean temperatures within an egg is too low for an embryo to alter its mean temperature by moving, it might

nonetheless be able to select a position with higher or lower diel variance in temperature (because subterranean clines in thermal variance are steeper than clines in mean temperature).

Interestingly, most of these points of agreement are apparent even in the first descriptions of the phenomenon; for example, Li, Zhao, Zhou, Hu, and Du (2014) note that behavioral thermoregulation by embryos will not be possible in small eggs. Any suggestion that the phenomenon occurs in all reptile species is a “straw man” and clearly inconsistent with the evidence. Given that broad consensus, where do the disagreements lie? They involve the generality of the ideas. Although the titles of papers by Telemeco et al. (2016) (“Reptile embryos lack the opportunity to thermoregulate by moving within the egg”) and Cordero et al. (2017) (“Reptile embryos are not capable of behavioral thermoregulation in the egg”) imply outright rejection of our hypothesis, the main text within those papers is less absolutist. For example, Telemeco et al. (2016) conclude that “reptile embryos will rarely have the capacity or opportunity to behaviorally thermoregulate by moving within the egg.” Cordero et al.’s (2017) paper concludes with the statement that “limitations render adaptive behavioral thermoregulation difficult ... for reptile embryos.” This issue of titles of papers being more absolutist than the actual conclusions of the work is a general phenomenon, rather than a specific criticism of the papers by Telemeco et al. or Cordero et al. For example, we note that the titles of our own papers on this topic (e.g., “Thermoregulatory behavior is widespread in the embryos of reptiles and birds”) are equally simplistic.

The disagreements, then, lie in the proportions of nest sites and species that fulfill the conditions needed for behavioral thermoregulation by embryos. The conditions that enable such thermoregulation might be common (Du & Shine, 2015; Li et al., 2014) or rare (Cordero et al., 2017; Telemeco et al., 2016). That debate will be more difficult to resolve than one where the opposing sides express diametrically different opinions about whether or not some phenomenon can happen at all. For example, how common does behavioral thermoregulation by embryos need to be for it to qualify as “important”? Many traits that are rare are nonetheless widely regarded as biologically significant (e.g., parthenogenetic reproduction, placental transfer of nutrients in viviparous reptiles: Blackburn, 2015; Kearney, 2003). Perhaps the strongest comparison comes with behavioral thermoregulation by adult reptiles. For an adult reptile to behaviorally thermoregulate, it needs a range of operative temperatures (e.g., patches of sun and shade) that are sufficiently close for it to move between them. That might translate into a scale of several meters for a large mobile reptile, as opposed to millimeters for an embryo. Does that larger scale mean that thermoregulatory opportunities for adult reptiles are ubiquitous? No. Many reptiles live in habitats or are active at times of day that restrict thermal heterogeneity to levels too low to allow behavioral thermoregulation. For example, thermal gradients are minimal in the ocean (e.g., for many sea snakes: see Shine, Shine, & Shine, 2003) and in dense tropical forests (where thermal homogeneity forces thermoconformity: Hertz, Huey, & Stevenson, 1993; Huey & Slatkin, 1976; Rummery, Shine, Houston, & Thompson, 1995). Nocturnal reptiles are active at a time when thermal heterogeneity is minimal; behavioral

thermoregulation may be impossible for many such animals, a high proportion of the world’s reptile fauna (Shine & Madsen, 1996).

In summary, there is immense subjectivity in the judgment of “importance.” If behavioral thermoregulation by embryos affects fitness-relevant traits of even a minority of reptile species, the topic is biologically significant.

1.2 | Lacking definitive evidence, what would we expect a priori?

Given the logistical obstacles to quantifying fitness consequences of thermoregulation by embryos in natural nests, are there other criteria we can use to evaluate the likely significance of this behavior? Many evolutionary biologists infer an adaptive benefit if they see organisms performing complex behaviors that cannot easily be ascribed to simple consequences of physics (e.g., Williams, 1966). The history of the study of adaptation includes many cases where traits were discovered, interpreted as non-adaptive byproducts of other processes, but later shown to have functional importance. The classic list of “vestigial organs” (like the human appendix) is an excellent example of that phenomenon. Once viewed as simple non-adaptive remnants of traits that had once been functional, most of those examples are now seen as functional in their own right (Randal Bollinger, Barbas, Bush, Lin, & Parker, 2007). Most adaptive behaviors were described long before their benefits for organismal fitness were confirmed.

Interestingly, many putatively “non-adaptive” traits, later found to be functional, are those that occur during early ontogeny. As a general proposition, we expect that embryos (like later life stages) will behave in ways that enhance individual fitness (Williams, 1966). In contrast, Cordero et al. (2017) suggests that many behaviors manifested by embryos are simply preparations for functionality during post-hatching life (and hence are adaptive, but with the fitness benefit accruing during post-hatching life). We contend that although embryos are physically unable to perform many of the behaviors exhibited by older conspecifics, recent literature documents a broad array of adaptive behavioral responses by embryos. What could an embryo do that might plausibly enhance its own fitness? We suggest the following list of activities:

1. **Feed**—Embryos in many viviparous species (e.g., elasmobranchs, caecilians, dipterans) feed in utero, on resources such as infertile ova, live siblings, and the uterine lining. They sometimes exhibit specific morphological adaptations to do so (e.g., teeth in caecilian embryos: Wake, 1976);
2. **Kill siblings to reduce competition for food**—for example, invertebrates (Harrath, Sluys, Zghal, & Tekaya, 2009; Thomsen, Collin, & Carrillo-Baltodano, 2014) and sharks (Gilmore, 1993);
3. **Regulate amniotic fluid volume by drinking**—Cordero et al. (2017) interpret sucking behavior pre-birth as nonfunctional, but this behavior plausibly adjusts fluid volumes among compartments of the oviductal package (i.e., moving fluid from the amniotic sac to the allantois), thereby enhancing offspring viability (El-Haddad, Desai, Gayle, & Ross, 2004);

4. **"Eavesdrop" on developmental rates of siblings**—enabling embryos to hatch synchronously with the rest of the clutch, thereby gaining fitness benefits associated with concurrent emergence from the nest, predator satiation, and so on (McGlashan, Spencer, & Old, 2012);
5. **Identify the optimal time to hatch based on external cues**—including diel cycles (Radder & Shine, 2006), drought stress (Newman, 1988), and imminent predation (Warkentin, 2011);
6. **Learn dialect of local songs**—by listening to the mother's vocal repertoire while they are still inside the egg (in birds: Dowling, Colombelli-Négrel, & Webster, 2016);
7. **Vocalize within the egg shortly before hatching**—to induce hatching in siblings and stimulate the adult female to open the nest (in crocodylians: Vergne & Mathevon, 2008) or to exhibit other parenting behaviors (in birds: Bolhuis & van Kampen, 1992; Reed & Clark, 2011; Rumpf & Nichelmann, 1993); and
8. **Reposition within the egg**—to behaviorally thermoregulate (reptiles: Du et al., 2011; birds: Li et al., 2014; Marasco & Spencer, 2015).

Returning to our earlier theme, many of these adaptive behaviors have been demonstrated in only a small number of species—and likely, are rare overall. That rarity does not diminish the logic of the a priori expectation that a complex behavior is likely to have evolved because it has enhanced the fitness of individuals displaying that behavior.

Strong evidence for an adaptive significance to some of these behaviors does not, of course, mean that all are adaptive. But it does argue for caution in dismissing potentially adaptive behaviors as accidents of developmental pathways. If there is a plausible adaptive benefit to doing something, we expect selection to favor that behavior. The only convincing argument against that proposition is if the constraints that preclude the behavior are fundamental and irreversible. Below, we argue that the constraints proposed as barriers to behavioral thermoregulation by reptile embryos (inability to move within the egg; lack of thermal heterogeneity within the egg), although important under some conditions, are not universal.

1.3 | Can embryos move about enough to modify their own temperatures?

Extensive data (dating back to the 1880s, as noted by Cordero et al., 2017) confirm that tetrapod embryos move around within their egg, albeit over a limited proportion of development. Unfortunately, we do not know the mechanism by which that movement occurs, and hence are skeptical of Cordero et al.'s assumption that mobility cannot occur until musculature is well developed (indeed, we recorded movement at stages 14 onward [Du et al., 2011], earlier than Cordero et al. infer that such movement is possible).

It is nonetheless clear that an embryo lacks locomotor capacity very early in development, and there is too little free space for the embryo to move around very late in development. As Cordero et al. (2017) note, however (see their Figures 1 and 2), embryos in the middle part of development—a critical time for the elaboration of thermally sensitive traits (Andrews, 2004)—are highly mobile, with substantial room to move. And even late in development, repositioning within the egg

(possible even for a large embryo) might confer fitness benefits by exposing a specific part of the body to optimal thermal regimes. Given the diverse suite of phenotypic traits modified by incubation regimes (see above), some parts of the body may be more sensitive to thermal regimes than others; and some traits may have more effect on fitness than others. For example, in the scincid lizard *Bassiana duperreyi*, incubation temperatures not only affect a hatchling's date of emergence, sex, size, body shape, locomotor performance, antipredator tactics, and activity level, but also modify its cognitive abilities and brain structures (Amiel & Shine, 2012; Amiel, Bao, & Shine, 2017; Shine & Harlow, 1996). By repositioning, an embryo could expose especially fitness-critical parts of the body to specific incubation conditions.

The distances over which embryos can move are far smaller than for adult reptiles, even expressed in terms of body lengths rather than absolute distances. Are those distances large enough to influence offspring viability? It is difficult to provide a general answer to that question because even tiny thermal differences might affect offspring phenotypes (Deeming, 2004). The limited scale of embryo movement must powerfully constrain potential benefits; but to argue that no such benefits are possible, the capacity to move must be well-nigh zero. All performance traits (e.g., speed, stamina, stride length, maximum ingestible prey size) have some maximum limit. A limited range in trait values does not mean that selection cannot optimize the trait in question. An upper limit to performance says nothing about the biological significance of trait variation; so the argument by Telemeco et al. (2016) and Cordero et al. (2017) is compelling only if the upper limit for embryonic repositioning is very close to zero. It is not, at least in the middle third of embryonic development (Cordero et al., 2017).

How much space within the egg is available for an embryo to move around at each stage of embryogenesis? In Figures 2 and 3 of their paper, Cordero et al. (2017) attempt to quantify this variable by plotting embryo size through the course of incubation. Because a full-term embryo takes up virtually all the space within its egg, the proportion of final embryonic volume achieved at each stage of development provides a measure of how much space is taken up by the embryo. The difference between embryo size at each point versus final embryo size offers a measure of "free space" within which the embryo could move. Unfortunately, the graphs in Cordero et al.'s (2017) figures use an inappropriate size metric. They rely on linear measurements, which increase isometrically with time through the incubation period. In contrast, a graph of embryonic mass through incubation is strongly curvilinear: embryos remain very small until most of development is complete. For example, Nagle, Plummer, Congdon, and Fischer (2003) reported that turtle embryos were only 34% of final mass when 65% of the way through incubation. Our data on Chinese turtles (Du et al., 2011) reveal a similar situation (35% of total mass at 65% of incubation). The same pattern is evident in the photographs provided by Cordero et al. (panel b of their Figure 3); embryo volume remains low until well into incubation. One result of that allometry is that a small increment in absolute body length increases mass (and volume) far more in a large embryo than a small one. In consequence, linear measures overestimate the volume of a developing embryo, and thus underestimate the amount of "free space" remaining within the egg. Because reptile embryos complete most of their differentiation

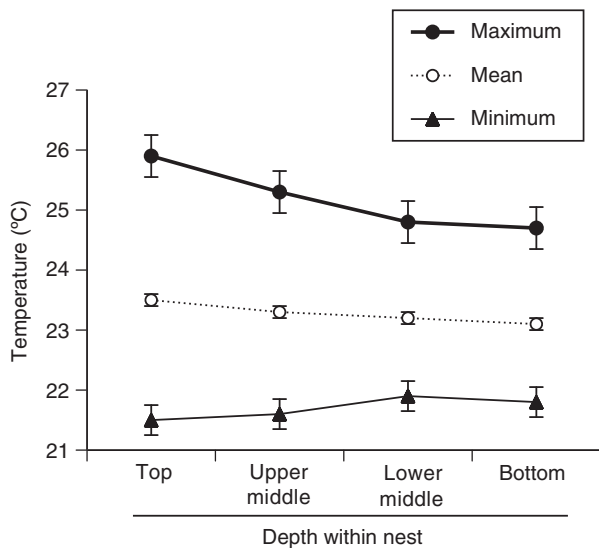


FIGURE 1 The effect of depth beneath the soil surface on thermal regimes experienced by embryos within natural nests of snapping turtles (*Chelydra serpentina*). The graph shows average daily values for the arithmetic mean temperature (central dotted line), and associated maximum (upper) and minimum (lower) temperatures. Data from Telemeco et al. (2016)

before embarking on a rapid increase in size at the end of incubation (Andrews, 2004), embryos in the middle part of incubation have most major anatomical systems in place while still having room to move.

1.4 | Is there enough thermal heterogeneity within an egg to allow embryos to thermoregulate?

The second major argument by Telemeco et al. (2016) and Cordero et al. (2017) is that thermal gradients are very small within most reptile eggs. Even in large eggs in shallow sun-exposed nests, temperatures within an egg are buffered by insulation from the eggshell and by circulation of heat within the internal fluids. We accept their argument (and applaud their careful measurement of the magnitude of such buffering effects), but are left with the same problem as above. For thermal variation available to an embryo to be so low that it precludes behavioral thermoregulation, that variation must be well-nigh zero. And demonstrably, that is not the case (Telemeco et al., 2016).

First, even a minor change in mean developmental temperature might be biologically significant. Critically, we do not know how large a thermal shift is needed to affect hatchling phenotypes. In some species, even a transitory period of cool weather during incubation has detectable impacts on offspring phenotypes many weeks later (Shine & Elphick, 2001). In species with temperature-dependent sex determination (TSD), the thermal shift needed to move from 100% male to 100% female offspring can be very narrow (e.g., Bull, 1980, 1985).

Second, although arithmetic mean temperature is relatively constant with increasing depth in a nest, thermal variance is not (Figure 1). The uppermost eggs in a nest experience strong diel cycles in temperature because the ground surface heats by day and cools by night. In deeper layers, the surrounding soil buffers those diel fluctuations (Telemeco et al., 2016). This basic prediction from soil biophysics has

been supported by many field studies on reptile nests (e.g., Ackerman & Lott, 2004; Packard, Tracy, & Roth, 1977, 1985). Those ubiquitous gradients mean that a small movement toward or away from the soil surface can expose an embryo to a more or less variable thermal regime. In a shallow sun-exposed nest, diel thermal variance is much higher closer to the ground surface than deeper down (based on data from Telemeco et al., 2016; ANOVA on their raw data, with depth beneath the soil as the factor [and nest as a random factor], shows that increasing depth beneath the soil surface did not change mean daily temperature [$F_{3,1232} = 2.22, P = 0.084$] but strongly shifted diel thermal range [$F_{3,1232} = 215.60, P < 0.0001$]; see Figure 1). Thus, despite buffering, the uppermost part of a large egg will experience a greater diel range of temperatures than does the lower part of that same egg. Telemeco et al. (2016) do not consider this aspect of thermal regimes, but Cordero et al. (2017) concede that behavioral thermoregulation potentially allows an embryo to select a position of higher versus lower thermal variance within the egg.

Although clines in thermal variance with depth below the ground surface are common, Cordero et al. (2017) suggest that embryos are unable to exploit those clines because they cannot track dynamically changing temperatures. We agree that a simple rule such as “select a hotter or cooler available place within the egg” (the only “rule” investigated in empirical work to date: Du et al., 2011; Li et al., 2014) will not enable an embryo to locate itself in the position with highest thermal variance. But an embryo can evolve a rule that says “select a place where temperature changes rapidly.” Such a rule does not require any capacity for long-term “memory” (contra Cordero et al., 2017); it simply requires detection of short-term rates of thermal change (or even, spatial variation in temperature that results from rapid thermal change). Optimal levels of thermal variance vary among sites and through time (as suggested by Cordero et al., 2017) but in many reptile populations, the optimal thermal variance for embryonic development may be consistently higher or lower than that available in all nest-sites in that region. Thus, selection could favor a consistent embryonic preference (e.g., for high thermal variance: Shine & Harlow, 1996). There is no need to invoke complex evaluation of variance optima.

If we accept that reptile embryos often will have access to sites within the egg that provide different levels of diel variance in temperature, could selection of higher thermal variance (for example) impact offspring fitness, independent of mean temperature? Laboratory studies unequivocally demonstrate that the phenotypic traits of hatchling reptiles (including body sizes, shapes, locomotor performance, and behavior) are influenced by thermal variance as well as by mean incubation temperature (e.g., Ashmore & Janzen, 2003; Patterson & Blouin-Demers, 2008; Shine & Harlow, 1996; Telemeco, Abbott, & Janzen, 2013). Offspring sex ratios in reptile species with TSD also are influenced by thermal variance as well as mean incubation temperature (turtles: Bull, 1980, 1983; Du, Shen, & Wang, 2009; Neuwald & Valenzuela, 2011; Schwarzkopf & Brooks, 1985; lizards: Warner & Shine, 2011). Thermal variance can affect offspring phenotypes directly (Georges, 1989; Georges, Beggs, Young, & Doody, 2005), and also by influencing the effective mean temperature for embryonic development (if developmental rates are non-linearly affected by incubation temperature, as is generally true for reptile embryos: Du &

Shine, 2010; Georges, 1989; Shine & Harlow, 1996; Telemeco et al., 2013; Yntema, 1978). Additionally, diel changes in temperature can affect the flow of water into and out of the egg, another potential influence on hatchling fitness (Ackerman & Lott, 2004). The effects of thermal variance on phenotypic traits of the hatchling vary considerably among species (e.g., Ji, Gao, & Han, 2007; Li, Zhou, Ding, & Ji, 2013a,b; Lin, Li, An, & Ji, 2008) and thermal variance may constitute a significant nest-site selection criterion for female reptiles (Shine & Harlow, 1996; Warner & Shine, 2008).

We also note that previous discussions on this topic (our own included) have made the simplifying assumption that any temperature-related advantage to an embryo moving to a specific point on a thermal gradient (as embryos do in laboratory experiments) is mediated via a shift in the body temperature of that embryo. Although we have no data to evaluate it, there is an alternative possibility; an embryo may enhance its fitness by moving close to a “hotspot” on the shell surface, even if that proximity does not affect its body temperature. For example, a hotter section of the external surface of the shell may influence the temperature of extra-embryonic membranes that are tightly applied to the underside of the shell, even if heat circulation within the egg minimizes thermal impacts on the embryo. Modifying the thermal environment of the embryo's life-support systems may influence fitness in ways that are not yet understood.

2 | CONCLUDING REMARKS

Both sides of the debate agree that many reptile species have attributes (such as small eggs, deep and/or shaded nests) that preclude embryonic control over thermal regimes. However, both sides also agree on the existence of temperature differentials (especially, in thermal variance) within reptile eggs in some natural nests; and agree that embryos during the middle part of development are capable of moving about within the egg, and have enough room to do so. Thus, an embryo in that situation can potentially select a thermal regime that enhances its fitness relative to that of a thermoconforming sibling.

Just because an embryo has the opportunity to thermoregulate does not mean, however, that it actually does so (or that there is any adaptive significance to the behavior). More generally, we need to clearly articulate the question we are asking. For example, the question “is movement by embryos adaptive?” is a far more general one than “do embryos exhibit behavioral thermoregulation?” Some of the suggestions by Telemeco et al. and Cordero et al. involve potentially adaptive functions for embryo movement that do not involve thermal factors. For example, adaptive advantages related to “play” or neural stimulation might be achieved by any kind of embryo movement. In contrast, our own papers have focused on the subset of embryo movements induced by experimentally imposed thermal gradients.

The potential adaptive significance of embryo movement (including “play” and neural stimulation as well as thermoregulation) remains unclear, and we need empirical studies to resolve this issue. Arguments from plausibility (such as the analyses by Cordero et al., 2017, and the current paper) can identify where to look, and what parameters to measure, but progress in understanding this topic ultimately will

come from studies in the laboratory and especially, in the field. The current debate has identified several questions that warrant further research—for example:

1. Do embryos move about in response to clines in thermal variance as well as in mean temperature?
2. Does embryonic behavioral thermoregulation enhance offspring fitness only during the middle part of development (when the embryo is able to move, and has room to do so)?
3. What is the minimum thermal differential (in terms both of mean temperature and thermal variance) required to significantly modify hatchling fitness?
4. What is the maximal speed at which an embryo can reposition itself within its egg in response to thermal cues?
5. Do physical attachments to the shell or to extra-embryonic membranes constrain an embryo's ability to move within the egg?
6. At what developmental stages can reptilian embryos move about, and using what mechanisms?
7. How strong and consistent are within-egg thermal differentials in natural nests of squamates and turtles (and what factors drive that variation)?
8. Do large embryos reposition themselves to expose specific parts of the body to optimal thermal regimes?
9. Given that eggs of many reptile species are too small or deeply buried for embryonic movement to modify the temperature of an embryo, why do the embryos of such species still move within the egg in laboratory experiments that impose thermal gradients?
10. Can proximity to a “hotspot” on the external surface of an egg influence the biology of an embryo, even if it does not modify its body temperature?
11. How can we directly measure embryonic movements in natural nests? and
12. Can we apply the widely-used indices of Hertz et al. (1993) to directly compare the thermoregulatory biology of embryos and of post-hatching conspecifics?

Ideally, we could answer many of these questions by direct manipulation of an embryo's ability to move, to experimentally test the benefits as well as costs of embryonic thermoregulation (Zhao, Ding, & Zhang, 2014).

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REFERENCES

- Ackerman, R. A., & Lott, D. B. (2004). Thermal, hydric and respiratory climate of nests. In D. C. Deeming (Ed.), *Reptilian Incubation: Environment, Evolution and Behavior* (pp. 15–43). Nottingham, UK: Nottingham University Press.
- Amiel, J. J., & Shine, R. (2012). Hotter nests produce smarter young lizards. *Biology Letters*, 8, 372–374.
- Amiel, J. J., Bao, S., & Shine, R. (2017). The effects of incubation temperature on the development of the cortical forebrain in a lizard. *Animal Cognition*, 20, 117–125.
- Andrews, R. (2004). Patterns of embryonic development. In D. C. Deeming (Ed.), *Reptilian Incubation: Environment, Evolution and Behavior* (pp. 75–102). Nottingham, UK: Nottingham University Press.
- Ashmore, G. M., & Janzen, F. J. (2003). Phenotypic variation in smooth soft-shell turtles (*Apalone mutica*) from eggs incubated in constant versus fluctuating temperatures. *Oecologia*, 134, 182–188.
- Blackburn, D. G. (2015). Evolution of vertebrate viviparity and specializations for fetal nutrition: A quantitative and qualitative analysis. *Journal of Morphology*, 276, 961–990.
- Bolhuis, J. J., & van Kampen, H. S. (1992). An evaluation of auditory learning in filial imprinting. *Behaviour*, 122, 195–230.
- Bull, J. J. (1980). Sex determination in reptiles. *Quarterly Review of Biology*, 55, 3–21.
- Bull, J. J. (1983). *Evolution of sex determining mechanisms*. Menlo Park, CA: Benjamin/Cummings Publishing Company.
- Bull, J. J. (1985). Sex ratio and nest temperature in turtles: Comparing field and laboratory data. *Ecology*, 66, 1115–1122.
- Cordero, G. A., Telemeco, R. S., & Gangloff, E. J. (2017). Reptile embryos are not capable of behavioral thermoregulation in the egg. *Evolution and Development*, 20, 40–47. <https://doi.org/10.1111/ede.12244>
- Deeming, D. C. (2004). Post-hatching phenotypic effects of incubation on reptiles. In D. C. Deeming (Ed.), *Reptilian incubation: Environment, evolution and behavior* (pp. 229–251). Nottingham, UK: Nottingham University Press.
- Dowling, J. L., Colombelli-Négrel, D., & Webster, M. S. (2016). Kin signatures learned in the egg? Red-backed fairy-wren songs are similar to their mother's in-nest calls and songs. *Frontiers in Ecology and Evolution*, 4, 48.
- Du, W-G., & Shine, R. (2010). Why do the eggs of lizards (*Bassiana duperreyi*, Scincidae) hatch sooner if incubated at fluctuating rather than constant temperatures? *Biological Journal of the Linnean Society*, 101, 642–650.
- Du, W-G., & Shine, R. (2015). The behavioral and physiological strategies of bird and reptile embryos in response to unpredictable variation in nest temperature. *Biological Reviews*, 90, 19–30.
- Du, W-G., Shen, J. W., & Wang, L. (2009). Embryonic development rate and hatchling phenotypes in the Chinese three-keeled pond turtle (*Chinemys reevesii*): The influence of fluctuating temperature versus constant temperature. *Journal of Thermal Biology*, 34, 250–255.
- Du, W-G., Zhao, B., Chen, Y., & Shine, R. (2011). Behavioral thermoregulation by turtle embryos. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 9513–9515.
- El-Haddad, M. A., Desai, M., Gayle, D., & Ross, M. G. (2004). *In utero* development of fetal thirst and appetite: Potential for programming. *Journal of the Society for Gynecologic Investigation*, 11, 123–130.
- 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., Beggs, K., Young, J. E., & Doody, J. S. (2005). Modelling development of reptile embryos under fluctuating temperature regimes. *Physiological and Biochemical Zoology*, 78, 18–30.
- Gilmore, R. G. (1993). Reproductive biology of lamnoid sharks. *Environmental Biology of Fishes*, 38, 95–114.
- Harrath, A. H., Sluys, R., Zghal, F., & Tekaya, S. (2009). First report of adelphophagy in flatworms during the embryonic development of the planarian *Schmidtea mediterranea* (Benazzi, Bagnà, Ballester, Puccinelli & Del Papa, 1975) (Platyhelminthes, Tricladida). *Invertebrate Reproduction and Development*, 53, 117–124.
- Hertz, P. E., Huey, R. B., & Stevenson, R. D. (1993). Evaluating temperature regulation by field-active ectotherms: The fallacy of the inappropriate question. *American Naturalist*, 142, 796–818.
- Huey, R. B., & Slatkin, M. (1976). Costs and benefits of lizard thermoregulation. *Quarterly Review of Biology*, 51, 363–384.
- Ji, X., Gao, J. F., & Han, J. (2007). Phenotypic responses of hatchlings to constant versus fluctuating incubation temperatures in the multi-banded krait, *Bungarus multicinctus* (Elapidae). *Zoological Science*, 24, 384–390.
- Kearney, M. R. (2003). Why is sex so unpopular in the Australian desert? *Trends in Ecology & Evolution*, 18, 605–607.
- Li, H., Zhou, Z. S., Ding, G. H., & Ji, X. (2013a). Fluctuations in incubation temperature affect incubation duration but not morphology, locomotion and growth of hatchlings in the sand lizard *Lacerta agilis* (Lacertidae). *Acta Zoologica*, 94, 11–18.
- Li, H., Zhou, Z. S., Wu, T., Wu, Y. Q., & Ji, X. (2013b). Do fluctuations in incubation temperature affect hatchling quality in the Chinese soft-shelled turtle *Pelodiscus sinensis*? *Aquaculture*, 406, 91–96.
- Li, T., Zhao, B., Zhou, Y. K., Hu, R., & Du, W-G. (2014). Thermoregulatory behavior is widespread in the embryos of reptiles and birds. *American Naturalist*, 183, 445–451.
- Lin, L. H., Li, H., An, H., & Ji, X. (2008). Do temperature fluctuations during incubation always play an important role in shaping the phenotype of hatchling reptiles? *Journal of Thermal Biology*, 33, 193–199.
- Marasco, V., & Spencer, K. A. (2015). Improvements in our understanding of behaviour during incubation. In D. C. Deeming & S. J. Reynolds (Eds.), *Nests, eggs, and incubation: New ideas about avian reproduction* (pp. 142–151). Oxford, UK: Oxford University Press.
- McGlashan, J. K., Spencer, R-J., & Old, J. M. (2012). Embryonic communication in the nest: Metabolic responses of reptilian embryos to developmental rates of siblings. *Proceedings of the Royal Society B*, 279, 1709–1715.
- Nagle, R. D., Plummer, M. V., Congdon, J. D., & Fischer, R. U. (2003). Parental investment, embryo growth, and hatchling lipid reserves in softshell turtles (*Apalone mutica*) from Arkansas. *Herpetologica*, 59, 145–154.
- Newman, R. A. (1988). Adaptive plasticity in development of *Scaphiopus couchii* tadpoles in desert ponds. *Evolution*, 42, 774–783.
- Neuwald, J. L., & Valenzuela, N. (2011). The lesser known challenge of climate change: Thermal variance and sex-reversal in vertebrates with temperature-dependent sex determination. *PLoS One*, 6, e18117.
- Packard, G. C., Tracy, C. R., & Roth, J. J. (1977). The physiological ecology of reptilian eggs and embryos and the evolution of viviparity within the Class Reptilia. *Biological Reviews*, 52, 71–105.
- Packard, G. C., Paukstis, G. L., Boardman, T. J., & Gutzke, W. H. (1985). Daily and seasonal variation in hydric conditions and temperature inside nests of common snapping turtles (*Chelydra serpentina*). *Canadian Journal of Zoology*, 63, 2422–2429.
- Patterson, L. D., & Blouin-Demers, G. (2008). The effect of constant and fluctuating incubation temperatures on the phenotype of black ratsnakes (*Elaphe obsoleta*). *Canadian Journal of Zoology*, 86, 882–889.
- Radder, R., & Shine, R. (2006). Thermally induced torpor in fullterm lizard embryos synchronizes hatching with ambient conditions. *Biology Letters*, 2, 415–416.

- Randal Bollinger, R., Barbas, A. S., Bush, E. L., Lin, S. S., & Parker, W. (2007). Biofilms in the large bowel suggest an apparent function of the human vermiform appendix. *Journal of Theoretical Biology*, *249*, 826–831.
- Reed, W. L., & Clark, M. E. (2011). Beyond maternal effects in birds: Responses of the embryo to the environment. *Integrative & Comparative Biology*, *51*, 73–80.
- Rummery, C., Shine, R., Houston, D. L., & Thompson, M. B. (1995). Thermal ecology of the Australian southern angle-headed dragon, *Hypsilurus spinipes* (Agamidae). *Copeia*, *1995*, 818–827.
- Rumpf, M., & Nichelmann, M. (1993). Development of prenatal acoustic interaction in the muscovy duck (*Cairina moschata*). *British Poultry Science*, *34*, 287–296.
- Schwarzkopf, L., & Brooks, R. J. (1985). Sex determination in northern painted turtles: Effect of incubation at constant and fluctuating temperatures. *Canadian Journal of Zoology*, *63*, 2543–2547.
- Shine, R., & Elphick, M. J. (2001). The effect of short-term weather fluctuations on temperatures inside lizard nests, and on the phenotypic traits of hatching lizards. *Biological Journal of the Linnean Society*, *72*, 555–565.
- Shine, R., & Harlow, P. S. (1996). Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology*, *77*, 1808–1817.
- Shine, R., & Madsen, T. (1996). Is thermoregulation unimportant for most reptiles? An example using water pythons (*Liasis fuscus*) in tropical Australia. *Physiological Zoology*, *69*, 252–269.
- Shine, R., Shine, T., & Shine, B. (2003). Intraspecific habitat partitioning by the sea snake *Emydocephalus annulatus* (Serpentes, Hydrophiidae): The effects of sex, body size, and color pattern. *Biological Journal of the Linnean Society*, *80*, 1–10.
- Telemeco, R. S., Abbott, K. C., & Janzen, F. J. (2013). Modeling the effects of climate change-induced shifts in reproductive phenology on temperature-dependent traits. *American Naturalist*, *181*, 637–648.
- Telemeco, R. S., Gangloff, E. J., Cordero, G. A., Mitchell, T. S., Bodensteiner, B. L., Holden, K. G., ... Janzen, F. J. (2016). Reptile embryos lack the opportunity to thermoregulate by moving within the egg. *American Naturalist*, *188*, E13–E27. <https://doi.org/10.1086/686628>
- Thomsen, O., Collin, R., & Carrillo-Baltodano, A. (2014). The effects of experimentally induced adelphophagy in gastropod embryos. *PLoS One*, *9*, e103366.
- Vergne, A. L., & Mathevon, N. C. (2008). Crocodile egg sounds signal hatching time. *Current Biology*, *18*, R513–R514.
- Wake, M. H. (1976). The development and replacement of teeth in viviparous caecilians. *Journal of Morphology*, *148*, 33–63.
- Warkentin, K. M. (2011). Environmentally cued hatching across taxa: Embryos respond to risk and opportunity. *Integrative and Comparative Biology*, *51*, 14–25.
- Warner, D. A., & Shine, R. (2008). Maternal nest-site choice in a lizard with temperature-dependent sex determination. *Animal Behaviour*, *75*, 861–870.
- Warner, D. A., & Shine, R. (2011). Interactions among thermal parameters determine offspring sex under temperature-dependent sex determination. *Proceedings of the Royal Society B*, *278*, 256–265.
- Williams, G. C. (1966). *Adaptation and natural selection: A critique of some current evolutionary thought*. Princeton, NJ: Princeton University Press.
- Yntema, C. L. (1978). Incubation times for eggs of the turtle *Chelydra serpentina* (Testudines: Chelydridae) at various temperatures. *Herpetologica*, *34*, 274–277.
- Zhao, B., Li, T., Shine, R., & Du, W-G. (2013). Turtle embryos move to optimal thermal environments within the egg. *Biology Letters*, *9*, 20130337.
- Zhao, B., Ding, P., & Zhang, W. (2014). Energetic cost of behavioral thermoregulation in turtle embryos. *Behavioral Ecology*, *25*, 924–927.

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