

# The behavioural and physiological strategies of bird and reptile embryos in response to unpredictable variation in nest temperature

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## ABSTRACT

Temperature profoundly affects the rate and trajectory of embryonic development, and thermal extremes can be fatal. In viviparous species, maternal behaviour and physiology can buffer the embryo from thermal fluctuations; but in oviparous animals (like most reptiles and all birds), an embryo is likely to encounter unpredictable periods when incubation temperatures are unfavourable. Thus, we might expect natural selection to have favoured traits that enable embryos to maintain development despite those fluctuations. Our review of recent research identifies three main routes that embryos use in this way. Extreme temperatures (*i*) can be avoided (e.g. by accelerating hatching, by moving within the egg, by cooling the egg by enhanced rates of evaporation, or by hysteresis in rates of heating *versus* cooling); (*ii*) can be tolerated (e.g. by entering diapause, by producing heat-shock proteins, or by changing oxygen use); or (*iii*) the embryo can adjust its physiology and/or developmental trajectory in ways that reduce the fitness penalties of unfavourable thermal conditions (e.g. by acclimating, by exploiting brief windows of favourable conditions, or by producing the hatchling phenotype best suited to those incubation conditions). Embryos are not simply passive victims of ambient conditions. Like free-living stages of the life cycle, embryos exhibit behavioural and physiological plasticity that enables them to deal with unpredictable abiotic challenges.

*Key words:* amniote, developmental plasticity, embryogenesis, thermoregulation, thermal acclimation.

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## I. INTRODUCTION

The embryo is a critical stage of the life history, not only because it can comprise a significant part of the entire lifespan (e.g. some chameleons spend more than 50% of their lives as embryos: Karsten *et al.*, 2008), but also because it is likely to be more vulnerable to external conditions (e.g. temperature, moisture, pathogens, pollution, etc.) than are post-hatching individuals (Deeming & Ferguson, 1991*a*; Jezierska, Ługowska & Witeska, 2009). In viviparous species, that vulnerability is reduced by maternal buffering of incubation conditions, but even so, many birth defects (with lifelong consequences) are attributable to disrupted early embryogenesis in humans (Ferretti *et al.*, 2006), and thermal conditions during pregnancy can affect offspring phenotypes in viviparous reptiles (Lourdais *et al.*, 2004; Ji *et al.*, 2006). The embryo's vulnerability to unpredictable fluctuations in external conditions is likely to be far greater in oviparous (egg-laying) animals, where embryos develop outside the mother's body and thus are more directly exposed to the external environment. The duration of embryonic life also may be important: in taxa such as amniote vertebrates that produce large eggs (that hence take a long time to develop prior to hatching: Iverson & Ewert, 1991; Shine, 2005; Deeming *et al.*, 2006), there is more opportunity for disruption by environmental conditions than is the case for the smaller (and typically, rapidly developing) eggs of amphibians and fishes (Gillooly *et al.*, 2002; Wells, 2007).

Given a long exposure to unpredictably variable incubation conditions, we might expect oviparous amniotes to have evolved a suite of adaptive responses that cushion any negative effects of such variation on embryogenesis, both in terms of embryonic survival to hatching and of the developmental trajectories that enhance fitness-relevant phenotypic traits of hatchlings (Deeming & Ferguson, 1991*a*). Some of those adaptive responses may involve maternal traits, such as judicious thermoregulation prior to oviposition (Mathies & Andrews, 1997), and careful nest-site selection such that embryos experience relatively stable, favourable conditions prior to hatching despite substantial (e.g. spatial or temporal) variation in external conditions (Doody *et al.*, 2006; Telemeco, Elphick & Shine, 2009). Less scientific attention has been focused on an alternative (complementary) route

for adaptive responses to variable incubation conditions: the possibility that embryos can respond to such variations by flexibly adjusting either the conditions they experience, or the way that those conditions impact on embryonic viability and development.

Despite the importance of the embryonic stage in any organisms' life cycle, the question of how embryos respond to their environments has remained largely unstudied, compared to the extensive literature on tactics of free-living stages of the life cycle to cope with fluctuating abiotic conditions (temperature, moisture, salinity, pH, etc.) (Willmer, Stone & Johnston, 2004; Angilletta, 2009). This bias largely reflects the traditional assumption that embryos are simply organisms-in-progress, and have little control over their own destinies until after hatching. However, recent studies on reptile and bird embryos have demonstrated that the embryos combine behavioural and physiological ways to control aspects of their own pre-hatching environment, showing surprisingly sophisticated and effective means of adapting to their environments (e.g. Andrewartha, Tazawa & Burggren, 2011; Du *et al.*, 2011). In this review, we examine behavioural and physiological tactics that enable embryos to evade or tolerate potentially deleterious incubation conditions. We focus on thermal variation (rather than, for example, variation in hydric conditions), because ambient temperature has profound and immediate effects on organismal function (Angilletta, 2009) whereas, for example, an embryo may be able to buffer short-term hydric challenges by storing water during earlier more favourable conditions (Brown & Shine, 2005). Our aim is to review embryonic responses to thermal challenges in reptiles and birds, and point out some future directions and potential challenges in this emerging research field.

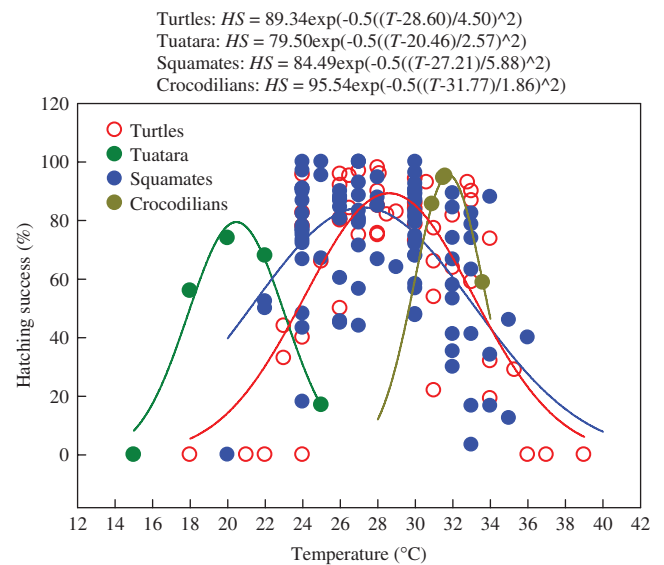
## II. THERMAL CHALLENGES FACING AN EMBRYO

The embryos of reptiles and birds experience considerable thermal variation at a range of temporal and spatial scales. In natural nests of both reptiles and birds, the temperatures experienced by embryos are unpredictable through space and time, depending upon factors such as local weather

conditions, the presence of shading vegetation, and the brooding behaviour of parents (Janzen, 1994; Ackerman & Lott, 2004; Jia, Sun & Swenson, 2010). Some of the variation (e.g. between seasons) is consistent enough to be broadly predictable, but large fluctuations can occur at time scales ranging from daily to seasonally (Thompson, 1988; Goth, 2007). In some types of nests (notably, shallow nests of reptiles in sun-exposed areas), the diel thermal fluctuation can be substantial, often greater than the divergence in mean thermal values among nests over a large area (Shine & Harlow, 1996; Shine, Elphick & Barrott, 2003). Embryos in such nests thus must cope with temperatures that vary dramatically through the diel cycle, as well as in response to stochastic weather events (Shine, 2002).

In reptiles, mean nest temperatures often fall within a range of about 20–30°C, with extreme temperatures ranging from approximately 10 to 45°C (Thompson, 1988; Ackerman & Lott, 2004). In the few species for which extensive data sets are available on incubation conditions (i.e. multiple nests in multiple sites, over multiple years), the total range of variation is considerable. For example, temperatures inside natural nests of the Australian skink *Bassiana duperreyi* have been reported to vary from 9 to 43°C (Shine & Harlow, 1996; Shine *et al.*, 2003). Nest temperatures are less variable in birds than in reptiles because of pervasive parent brooding. Nonetheless, nest temperatures vary from about 30 to 40°C among avian species (Webb, 1987), and can drop to as low as 10°C for several hours daily if brooding parents leave to forage (Lill, 1979; Jia *et al.*, 2010). Thermal environments may even differ among different layers in a large clutch of eggs, or between the centre and periphery of the nest, due to disparities in proximity to the sun-warmed soil surface in reptiles and the brood patch in birds, or to metabolic heat produced by embryonic metabolism of adjacent eggs (Caldwell & Cornwell, 1975; Thompson, 1988; Zbinden, Margaritoulis & Arlettaz, 2006; Boulton & Cassey, 2012). For example, thermal differentials of up to 6°C between eggs occur within field nests of the freshwater turtle *Emydura macquarii*, and up to 9°C in the Australian brush-turkey *Alectura lathami* (Thompson, 1988; Goth, 2007). Lastly, temperatures may vary even within a single egg. In turtle nests, the end of an egg closest to the sun-warmed soil surface may be (at least transiently) up to 3°C hotter than the other end of the same egg (Zhao *et al.*, 2013).

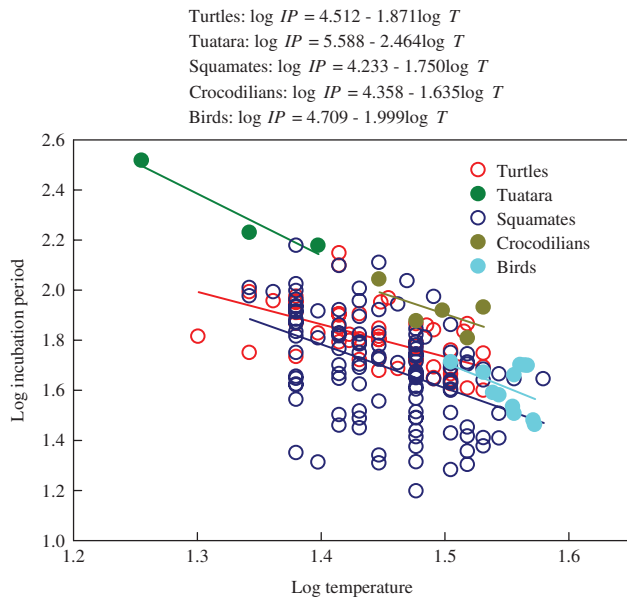
Incubation temperature has profound effects on a range of fitness-relevant traits. Some of those traits relate to embryonic development *per se* (e.g. metabolic rate, incubation duration and hatching success) whereas others relate to hatchling phenotypes (e.g. morphology, locomotor performance, behaviour, growth and gender) in reptiles (Janzen & Paukstis, 1991; Deeming & Ferguson, 1991b; Deeming, 2004; Booth, 2006) as well as in birds (DuRant *et al.*, 2013). The most obvious potential impact of extreme thermal variation is to exceed embryonic thermal tolerances, such that embryos die if exposed to overly high or low temperatures. Thermal tolerances for normal development differ among phylogenetic lineages both in reptiles and in



**Fig. 1.** Thermal dependence of hatching success ( $HS$ ) in reptile embryos. Data on hatching success were taken from published literature on one species of tuatara, eight species of turtles, 26 species of lizards and snakes, and two species of crocodilians (see online Table S1). Hatching success of reptile embryos was plotted against incubation temperature ( $T$ ) to determine the thermal dependence of embryonic survival; a three-parameter Gaussian peak function was used to fit the curve separately for each lineage of tuatara, turtles, squamates and crocodilians. Strong divergences are apparent – for example, optimal incubation temperature is much higher in crocodilians than in the tuatara.

birds. Most reptile embryos cannot develop successfully when incubated at temperatures lower than 20°C or higher than 35°C, with divergent optimal development temperatures for different major lineages (i.e., tuatara, squamates, turtles and crocodilians: see Fig. 1 and online Supporting Information, Table S1). By contrast, most bird embryos can tolerate several hours' exposure to 36–39°C (Webb, 1987). Embryos of a few species can tolerate even more extreme thermal conditions. For example, eggs of the desert lizard *Dipsosaurus dorsalis* can hatch successfully at 38°C (Muth, 1980). Most penguins, which breed in cold areas, have a lower optimum incubation temperature (approximately 32.5°C) than do other birds (ca. 38°C: Webb, 1987).

Even if the incubation regime falls within the range that allows successful hatching, temperature can massively affect other important aspects. Most notably, higher temperatures reduce incubation period in both reptiles and birds. The slope of thermal dependence differs among phylogenetic lineages, as do overall mean incubation periods (tuatara > crocodiles > turtles > squamates: see Fig. 2 and online Table S1). Field studies have shown a strong link between hatching date and hatchling survival in a range of reptiles and birds (Spear & Nur, 1994; Warner & Shine, 2007), so that more rapid development may confer significant benefits. Incubation temperature also can affect the energy-efficiency of embryogenesis, with larger hatchlings (relative



**Fig. 2.** Thermal sensitivity of incubation period ( $IP$ ) in reptile and bird embryos. Data on incubation periods were taken from published literature on one species of tuatara, 10 species of turtles, 34 species of lizards and snakes, two species of crocodylians, and three species of birds (see online Table S1). Data on incubation period and temperature ( $T$ ) were log-transformed, and linear regression was used to analyze the relationship between embryonic developmental time and incubation temperature in each lineage of reptiles and birds. Temperature had broadly similar effects in all lineages, despite major differences in overall mean incubation periods among groups.

to initial egg size) from some thermal conditions than others (Angilletta, Winters & Dunham, 2000; Ji & Du, 2001a). This, in turn, may reflect a lower thermal threshold for embryonic survival than for embryonic development. Below the threshold of development, embryos continue to use energy for maintenance activities but do not achieve substantial morphological change (Du *et al.*, 2009). Within the range of incubation temperatures that allows viable development, the thermal regime also may induce significant variation in hatchling phenotypes, which in turn influence the post-hatching growth, survival and even future reproduction of offspring in both reptiles and birds (Ji *et al.*, 2003; Warner & Shine, 2007, 2008; DuRant *et al.*, 2010).

Clearly, then, the thermal regime that an embryo experiences, and the ways in which it can modify or respond to that variation, are likely to have significant consequences for the viability of that individual. Broadly, we might expect two types of adaptive responses to those challenges: one to predictable conditions, and one to unpredictable conditions. The first of these pathways would be for a species (or population) to evolve relatively hard-wired (canalized) responses that fine-tune embryonic biology to the thermal conditions likely to be experienced in the nest. That process probably explains the examples of interspecific variation in thermal optima and tolerances for incubation that we

reference above: for example, tolerance of high temperatures in the eggs of desert lizards, and low temperatures in the eggs of penguins. However, our focus in the current review is on the second pathway by which embryos might deal with thermal variation: that is, flexible adjustments rather than canalized (fixed) traits. Because nest temperatures are highly dependent upon ambient conditions, and those conditions change unpredictably from place to place, hour to hour, day to day, week to week and year to year (Ackerman & Lott, 2004), we might expect embryos to be under strong selection to deal with such challenges. So what can an embryo do if the nest temperature changes unpredictably to become either colder or hotter than is optimal?

### III. THE POTENTIAL TACTICS AVAILABLE TO AN EMBRYO

Embryos are not passive entities. They may be able to respond to temporal and spatial variations in ambient temperature in many ways, at levels from the whole organism to the molecular. Broadly, we can distinguish three major pathways of response: the embryo can avoid, tolerate, or adjust to the unpredictable thermal variation that it encounters during development (Fig. 3). Below, we discuss each of these pathways in turn.

#### (1) The embryo can influence the temperature to which it is exposed

An embryo can influence its own developmental temperature in two ways: by reducing the amount of time that it spends in the nest (i.e. either by delaying oviposition or accelerating hatching), or by changing its own temperature within the nest (by affecting the overall temperature of its egg, its position within the egg, its rate of heating or cooling, or parental incubation behaviour).

##### (a) *Delaying the time (and stage of embryonic development) when the embryo moves from the mother's body into the nest*

An embryo might be able to affect its exposure to nest temperatures (and thus, its own developmental temperature) by influencing the time of oviposition. The time (and embryonic stage) at which eggs are laid are influenced by maternal traits, but may also be under embryonic control to some degree. Most reptiles and birds lay their eggs in late spring and summer, when ambient temperatures are suitable for embryonic development (Welty, 1982; Deeming, 2002, 2004). In at least some species, unsuitable environmental conditions can induce females to retain eggs rather than deposit them in nests that are suboptimal for embryonic development (Andrews & Rose, 1994; Buhlmann *et al.*, 1995). For example, experimental data show that female scincid lizards (*B. duperreyi*) kept under cool conditions retain their eggs *in utero*, eventually laying them when the embryos have reached a later embryonic stage (Telemeco *et al.*, 2010).



**Fig. 3.** Potential pathways by which an embryo may be able to respond to unpredictable thermal challenges within the nest in reptiles and birds.

Similarly, low environmental temperatures induce delayed oviposition in the agamid lizard *Calotes versicolor*; in this case, the growth of oviductal embryos is arrested (Shanbhag, Saidapur & Radder, 2003). Other environmental factors such as drought also may induce retention of eggs in female reptiles (e.g. Andrews & Rose, 1994; Mathies & Andrews, 1996).

(b) *Accelerating the time of hatching*

Another way to avoid disadvantageous incubation conditions is to hatch 'prematurely'. That is, embryos that are exposed to unsuitably low temperatures may emerge earlier from the nest, by modifying either developmental rate relative to temperature, or the stage of phenotypic differentiation required prior to hatching (Shine & Olsson, 2003; Doody, 2011). In an alpine scincid lizard, *B. duperreyi*, low nest temperatures late in the incubation period fail to retard hatching (Shine, 2002). Instead, the embryos hatch prematurely, and these 'premature' hatchlings exhibited reduced performance (e.g. low locomotor speeds, slow righting response), perhaps due to incomplete development of the musculoskeletal system or neural pathways controlling movement (Shine & Olsson, 2003). Reptile embryos also may delay (rather than accelerate) hatching in response to adverse environmental conditions, but this response appears to be related to hydric rather than thermal challenges (Ewert, 1991; Rafferty & Reina, 2012).

(c) *Redistributing water within the egg to increase evaporative cooling*

As reptile eggs develop, they accumulate water reserves from the surrounding nest substrate. Even brief periods of surplus water in the nest can allow an egg to take up water, for

later use to sustain embryogenesis even if the nest dries out substantially (Brown & Shine, 2005). Plausibly, an embryo may have some control over the distribution of water within the egg; and if exposed to potentially lethal temperatures, could selectively move water towards the eggshell to enhance evaporative water loss. We know of no published data to test this hypothesis, but unpublished work suggests that at least for large eggs, it may be important (Ming-Chung Tu, personal communication).

(d) *Moving within the egg to select or avoid hotspots*

The embryos of some reptile and bird species are capable of regulating their body temperature behaviourally. That is, an embryo responds to a thermal gradient within its egg (created by heating the egg from one end only) by moving towards the heat source (if mean egg temperature is suboptimal) or away from the heat source (if the 'hotspot' is potentially lethal) (Du *et al.*, 2011; Zhao *et al.*, 2013). Clearly, such thermal differentials are unlikely to occur in species with small eggs, or with deep nests where diel changes in solar radiation on the ground surface have little impact on nest temperatures. Nonetheless, we have now documented active thermoregulatory movements in the embryos of snakes, turtles, and birds, genuinely analogous to the thermoregulatory behaviour exhibited by post-hatching ectotherms (Du *et al.*, 2011; Zhao *et al.*, 2013, T. Li, B. Zhao, Y. K. Zhou, R. Hu & W. G. Du, unpublished data). For example, in the Chinese soft-shelled turtle (*Pelodiscus sinensis*), embryos are able to detect thermal differentials within the egg, and reposition themselves to exploit small-scale heterogeneity: they move to locally warm conditions both in laboratory experiments and in semi-natural nests (Du *et al.*, 2011). In

an emydid turtle (*Chinemys reevesii*), embryos actively seek out optimal thermal environments for their development (move away from dangerously high temperatures as well as towards warm temperatures), as do post-hatching individuals (Zhao *et al.*, 2013). In birds, embryos not only can move inside the egg to regulate their own body temperatures (T. Li, B. Zhao, Y. K. Zhou, R. Hu & W. G. Du, unpublished data), but also can adjust their thermal environment by using vocalizations to elicit more attentive incubation and solicit heat from parents (Evans, 1990; Brua, Nuechterlein & Buitron, 1996).

(e) *Hysteresis in rates of heating and cooling*

Embryos of some reptile species can influence their own rates of heating and cooling by physiological means. Live eggs heat up more rapidly than they cool down, whereas dead eggs and inanimate models tend to cool more rapidly than they heat (Du *et al.*, 2013). Cardiovascular flexibility is likely to play a critical role in controlling the heating and cooling rates, as it does in adult reptiles (Grigg, Drane & Courtice, 1979; Zaar, Larsen & Wang, 2004). However, the hysteresis in heart rates during heating *versus* cooling in embryos is in the opposite direction to that seen in adults (Du *et al.*, 2013), in which heart rates are higher during heating than cooling, allowing sun-warmed blood in superficial vessels to be deployed to functionally important parts of the animal's body (Bartholomew, 1982; Zaar *et al.*, 2004). The cause of this discrepancy of heart rate hysteresis between embryos and adults remains unclear, but might involve the different thermal environments facing embryos and post-hatching individuals.

(f) *Influencing parental incubation behaviour*

In birds, embryos may avoid stressful temperatures by eliciting adjustments in parental incubation behaviour. Because heat is lost as it is exchanged between the parental brood patch and eggs, embryo temperatures are rarely raised to the core body temperature of the adult bird (Deeming, 2008). Low ambient and egg temperatures may influence avian incubation behaviour, increasing nest attentiveness and decreasing off-bout duration (Franks, 1967; Drent, Postuma & Joustra, 1970; Conway & Martin, 2000). As heat flux from eggs increases in older embryos, nest-attending parents correspondingly increase egg cooling rates by altering incubation rhythms (short and frequent off-bouts) (Cooper & Voss, 2013). The communication avenue between embryos and their parents may involve nitric oxide. Embryos of several bird species emit nitric oxide through the shell during the second half of incubation, potentially inducing changes in blood circulation in the brood patch, and therefore in egg temperature (Ar & Sidis, 2002).

**(2) Modifying embryo physiology to allow toleration of thermal extremes**

If an embryo cannot influence its own temperature, it may nonetheless be able to modify its own physiology in ways that enhance its ability to tolerate the thermal extremes

that it encounters. The mechanisms involved likely differ depending upon whether the thermal threat involves overly high or overly low temperatures.

(a) *Cold torpor and embryonic diapause*

Low temperatures may disrupt embryonic function more if an embryo is developing than if it is in diapause (Ewert, 1991). Thus, embryos may avoid some of the negative effects of low temperature *via* developmental arrest [cold torpor, embryonic diapause; (Rafferty & Reina, 2012)]. Cold torpor is a facultative suspension of development that enables embryos to survive brief periods of temperatures that are too low to allow development, and can occur at any embryonic stage for most species of reptiles and birds during egg incubation (Ewert, 1991; Rafferty & Reina, 2012). In reptiles, embryos in freshly laid eggs can withstand days to weeks in cold torpor, whereas full-term embryos may use cold torpor to avoid hatching under disadvantageous (cold) environmental conditions (Christian, Tracy & Porter, 1986; Ewert, 1991; Radder & Shine, 2006). In birds, cold torpor is common at the beginning of incubation, with the embryos of earlier eggs in a clutch arresting development until all eggs in the clutch have been laid (Welty, 1982). Throughout the incubation period, cold torpor can be induced whenever eggs cool after the brooding parents leave to forage (Ewert, 1991). Post-oviposition embryonic diapause also occurs during winter in some reptilian species that lay eggs during autumn (e.g. chameleons, kinosternid turtles), but not in most birds and reptilian species that nest during spring and summer, when nest temperatures are suitable for embryonic development (Ewert, 1991; Andrews, 2004; Andrews *et al.*, 2008; Rafferty & Reina, 2012).

(b) *Heat shock proteins*

Embryos can tolerate extremely high temperatures in field nests of both reptiles and birds, at least for short periods (Webb, 1987; Shine *et al.*, 2003). Heat shock proteins (HSPs) are likely to play an important role in the heat tolerance of embryos, because HSPs function as molecular chaperones that help organisms to cope with thermal stress (Sørensen, Kristensen & Loeschcke, 2003). However, the role of HSPs in the heat tolerance of amniote embryos is largely unstudied. Our unpublished data (J. Gao, W. Zhang, W. Dang, Y. Mou, Y. Gao, B. J. Sun & W. G. Du, unpublished data) suggest that reptile embryos begin high-level expression of *Hsp70* at lower temperatures than do bird embryos; and the increase of *Hsp70* expression in response to heat stress is lower in reptile embryos than bird embryos, corresponding to lower heat tolerance in reptile embryos compared to bird embryos (see above).

(c) *Changes to oxygen supply and utilization*

The hypothesis of oxygen- and capacity-limited thermal tolerance (OLTT) suggests that whole-organism thermal tolerance in ectotherms is determined by temperature-dependent

limitations on oxygen supply and demand (Pörtner & Knust, 2007; Pörtner, 2010). Thus, an embryo might be able to improve its thermal tolerance by increasing its oxygen supply and/or changing the properties of its respiring mitochondria (e.g. mitochondrial density and enzyme activity). Reptile and bird embryos can facultatively adjust cardiac function, vascular density, and hematocrit and other blood parameters to enhance oxygen supply and cope with hypoxic conditions, especially in species with parchment (and thus, highly gas-permeable) eggshells (Corona & Warburton, 2000; Crossley & Altimiras, 2005; Du, Thompson & Shine, 2010a). Our unpublished data show that turtle embryos incubated at different temperatures differ in mitochondrial density and respiration, and in the activities of cytochrome *c* oxidase and lactate dehydrogenase (which play an important role in electron transport chain and anaerobic glycolysis) (B. J. Sun, T. Li, S. R. Li, J. Gao & W. G. Du, unpublished data). These results suggest that embryos can enhance their thermal tolerance by changing mitochondrial function, as do adult ectotherms (Fangue, Richards & Schulte, 2009).

### (3) Change embryo physiology so that it develops at an optimal (or near-optimal) rate despite challenges imposed by local thermal conditions

#### (a) Acclimation of developmental rate

Embryos can use thermal acclimation of metabolism to buffer the physiological consequences of nest temperature variation, in the same way as occurs for metabolic acclimation by post-hatching animals (see review by Huey & Berrigan, 1996; Angilletta, 2009). Metabolic compensation at low temperatures has been shown in the embryos of several species of reptiles (including a turtle *Chelydra serpentina*, a lizard *Sceloporus undulatus*, and a crocodile *Crocodylus johnstoni*, as well as in birds like the chicken *Gallus gallus*), as indicated by the lack of a significant difference in oxygen consumption rate between embryos incubated at high *versus* low temperatures during the second half of incubation (Whitehead, 1987; Tazawa *et al.*, 1988; Birchard & Reiber, 1996; Angilletta *et al.*, 2000). Recently, Du *et al.* (2010d) demonstrated that thermal acclimation of embryonic heart rate (metabolic rate) is widespread in reptiles; incubation at low temperatures induced embryos of turtles, lizards and snakes to increase their heart rates at any given test temperature. The study also identified at least one exception to this rule, however: in an Australian skink (*B. duperreyi*) whose natural nests experience dramatic diel thermal variation, the embryos did not acclimate their heart rates to mean incubation temperature.

Thermal acclimation may involve different pathways in different species, or even in different populations of a single species. For example, shifts in thermal acclimation and metabolic rate in high-latitude populations of a widespread lizard, *Sceloporus undulatus*, are responsible for a decrease of 13% in incubation period as compared to a conspecific low-latitude population (Du *et al.*, 2010c). Intriguingly, the two short-incubation period populations in *S. undulatus*

achieve their briefer incubation by different mechanisms: increased embryonic heart size in one population, and increasing heart rates in the other. The end result is the same: increased cardiac output enables higher metabolic and thus developmental rate (Du *et al.*, 2010c). The relative roles of canalized *versus* facultative responses to incubation temperature are unclear in this example, and warrant further study.

Acclimatory compensation of metabolic rate seems likely to be common in embryos, plausibly reflecting substantial selective advantages to maintaining rapid development even if eggs are exposed to suboptimal (low) temperatures in the nest. Earlier hatching often increases the survival rate of reptile hatchlings (Olsson & Shine, 1997; Warner & Shine, 2007; Wapstra *et al.*, 2010), favouring any acclimation system that can accelerate development of reptile embryos at low incubation temperatures. The shorter incubation period due to thermal acclimation also may reduce the risk that an embryo will be exposed to hazards such as extreme temperatures, desiccation and/or predation (Bobyn & Brooks, 1994).

#### (b) Synchronizing hatching with favourable abiotic conditions

Although some turtles spend long periods inside the nest after hatching, most reptiles emerge from the nest soon after breaking out of the eggshell (Gutzke, Paukstis & Packard, 1984). The fitness consequences of emergence under the wrong conditions – i.e. at a time of day or under weather conditions that encourage predator activity or reduce hatchling locomotor performance – may be severe. Perhaps reflecting these pressures, near full-term lizards (*B. duperreyi*) show a shift in the thermal dependence of heart rate, such that their activity is strongly suppressed at low temperatures (Radder & Shine, 2006). This mechanism may ensure that the lizards are active, and hence emerge from the nest, only under warm conditions (Radder & Shine, 2006). In turtles, eggs in upper and lower layers within a nest experience different thermal environments, but can achieve hatching synchrony by various proximate mechanisms. In an Australian freshwater turtle (*Emydura macquarii*), eggs that have developed at low temperatures respond to the proximity of more developed eggs (those incubated at higher temperatures) by increasing metabolic (developmental) rates and thus hatching earlier (McGlashan, Spencer & Old, 2012). By contrast, in the painted turtle (*Chrysemys picta*), embryos from the less-developed eggs hatch at an earlier stage of development (Colbert, Spencer & Janzen, 2010).

#### (c) Adjust developmental trajectories to local thermal conditions

Another adaptive response to suboptimal incubation conditions may be to modify phenotypic attributes of the hatchling in ways that minimize the fitness costs of those suboptimal conditions. The best example of this comes from studies of sex determination, whereby many reptiles (the tuatara, all crocodylians, and many turtles and lizards) determine offspring sex based on nest temperature

(Valenzuela & Lance, 2004). Theoretical models (supported by experimental studies: Warner & Shine, 2008) suggest that the adaptive significance of this tactic lies in producing the sex of offspring that can develop most effectively under local nest conditions: for example, a cooler nest may produce an adequate male but a very inferior female (Elphick & Shine, 1999). Thus, even in species where sex is usually determined by heteromorphic sex chromosomes, extreme incubation temperatures can override the chromosomal mechanism to reverse offspring sex (Shine, Elphick & Donnellan, 2002; Quinn *et al.*, 2007; Radder *et al.*, 2008).

(d) *Accelerate developmental rate under fluctuating temperatures*

One of the most common thermal challenges for reptile embryos in shallow nests is the high diel range in temperature. That challenge also provides an opportunity, because an embryo may be able to develop more rapidly at fluctuating temperatures than at constant temperatures (even if the mean temperature is the same), if embryonic cardiac output is increased more by a diel increase in temperature than it is decreased by a reduction in temperature. The asymmetry reflects the exponential relationship between heart rate (and thus, metabolic rate and developmental rate) and temperature (Du & Shine, 2010). Hence, shifts in the allometry of heart rate *versus* temperature can allow an embryo to exploit transient periods of high temperature within the nest to accelerate its development. The advantage disappears after hatching, and (as expected under this hypothesis), the allometry of heart rate *versus* temperature also shifts rapidly at the time of hatching in turtles Du *et al.*, (2010e).

#### IV. FUTURE PERSPECTIVES

How an embryo responds to its thermal environment is an emerging research field in ecology. Many gaps need to be filled both in terms of fundamental data and more sophisticated theoretical analyses on the thermal responses of embryos. Below, we point out some important directions and challenges for future studies on the thermal responses of animal embryos.

##### (1) Thermal environment of embryos

Given the difficulty of locating field nests of most reptiles, few data are available on the temperatures inside those nests, and even fewer on issues such as thermal differentials within eggs. Similar data on the embryonic thermal environment are also scarce for birds, despite the high visibility of bird nests. Thermal differentials within turtle eggs in a simulated nest may reach up to 3°C (Zhao *et al.*, 2013), but we know little about the thermal environment of embryos in nature. For example, how do thermal differentials within eggs differ as a function of egg size, distribution within the nest, or nest attributes? How do thermal differentials within eggs and within nests change temporally and spatially?

These questions provide exciting research opportunities and would substantially extend our understanding of the thermal challenges and opportunities encountered by embryos, as well as the ways in which these organisms respond to those challenges and opportunities.

##### (2) Behavioural and physiological responses

Our review confirms that embryos use a variety of behavioural and physiological tactics to cope with the complex thermal environment within a nest, but the mechanisms underlying these phenomena are largely unknown.

For example, how can a tiny embryo manage to move about within the egg? That embryonic movement is due to active thermoregulation, rather than passive embryonic repositioning caused by local heat-induced changes in viscosity of fluids within the egg, is clear because live embryos move towards a heat source whereas dead ones do not (Zhao *et al.*, 2013). The embryo's ability to move surely must occur after the development of neural and muscular systems that enable the embryo to detect heat and generate movement. Some amphibian embryos twist to reposition themselves within the eggs (Xiong *et al.*, 2010). Is the heat-seeking behaviour of reptile and bird embryos dependent on the same kinds of movement as used by these amphibian embryos? In addition, although we have demonstrated that embryos can physically reposition themselves within eggs to exploit minor thermal gradients (Du *et al.*, 2011), it is unclear if this repositioning can occur rapidly enough for an embryo to track diel fluctuations in temperature within a nest. Further studies on these topics would provide novel insights into the physiological basis of behavioural thermoregulation by embryos.

Second, why do reptile embryos (unlike adult reptiles) exhibit higher heart rates during cooling not heating (Du *et al.*, 2013)? We suggest the following hypothesis. In an adult reptile, the critical organs lie deep within the body; thus, a faster heart rate during heating increases the rate of heat flow from the sun-warmed body surface to those internal organs. By contrast, the embryo sits close to the surface of the egg, above a large metabolically inert yolk mass. As the external temperature increases, a slower heart rate may keep the warmest blood near the embryo, by reducing the rate at which heat (coming through the shell) is redirected into the egg's interior. The reverse process occurs during cooling: an adult reptile benefits by reducing the rate of heat flow from the core (internal organs) to the periphery, whereas an embryo benefits by increasing the rate of heat flow from the core (yolk) to the periphery (the embryo itself). Alternatively, slower heart rates might allow for increased stroke volume, and hence greater blood flow. To affect embryo temperatures substantially, shifts in heart rate likely would need to be combined with other mechanisms, such as countercurrent heat exchange or redirection of blood flow through shunting (Bartholomew, 1982; Zaar *et al.*, 2004). However, any redirection of blood away from the chorioallantois would reduce rates of gas exchange as well as heat exchange, and the trade-off between those two processes might well constrain the effectiveness of thermally



related cardiovascular shifts. No data are available on these topics for embryonic reptiles, but avian embryos are known to be capable of major chorioallantoic shunting of blood flow (Piper *et al.*, 1980).

Third, the thermal environments encountered by embryos will differ dramatically among species, and even among populations within wide-ranging species. For example, embryos of species that live in the temperate zone are likely to experience more variable nest temperatures (over a range of temporal scales) than will tropical species. Consequently, of thermoregulation and thermal acclimation abilities may be more advantageous for species in the temperate zone than the tropical zone. Empirical studies on populations of wide-ranging species could usefully assess whether or not thermoregulation and thermal acclimation are more prevalent in high-latitude populations than low-latitude populations. In addition, it would be of great interest to study the thermal response of embryos in other lineages than reptiles and birds. For example, some amphibians lay eggs in cold water in early spring (Wells, 2007); what physiological mechanisms allow the successful development of amphibian embryos at these low temperatures?

### (3) Biochemical basis of thermal response

Although living systems are based on a common set of biochemical structures and processes, and subject to a common set of physical–chemical laws, thermal adaptation at the individual level is paired with adaptive diversification at the biochemical level. Studies on adult animals have demonstrated that biochemical adaptation in response to temperature may involve changes in the composition of membranes, the activity of metabolic enzymes, and heat shock proteins (Johnston & Bennett, 1996; Hochachka & Somero, 2002). Animal embryos may use the same (or similar) biochemical mechanisms to cope with temperature change, but no such studies have been carried out on reptile or bird embryos.

### (4) The response of embryos to climate change

Published predictions of species responses to climatic change are exclusively based on the thermal biology of adult organisms (Huey *et al.*, 2009; Kearney, Shine & Porter, 2009). However, embryos also may be able to respond actively to environmental change. Accordingly, thermal adaptations of embryos may exacerbate or alleviate the negative effects of climate change inferred on the basis of adult thermal biology. To predict the response of embryos to climate change, we need to understand (i) relationships between the thermal biology of embryos and climate patterns on a global scale; (ii) how embryos respond to climate change, or buffer the effect of climate changes *via* behavioural and physiological adaptations, and (iii) how we can predict the future (evolved) responses of embryos to climate change. Macrophysiology, a unification of evolutionary physiology and macroecology, provides a wealth of theoretical and practical methods to identify local and global patterns of thermal adaptation

of embryos and to elucidate the underlying evolutionary processes (Chown & Gaston, 2008; Gaston *et al.*, 2009; Andrews & Schwarzkopf, 2012). Biophysical models (e.g. mechanistic models) may further help to predict embryonic responses to climate change, once we gather large enough datasets on the thermal biology of embryos (Kearney & Porter, 2009; Sinervo *et al.*, 2010).

## V. CONCLUSIONS

(1) Traditionally, embryos have been regarded as incapable of affecting their own incubation conditions. Contrary to that view, recent studies have demonstrated that reptile and bird embryos exhibit sophisticated and effective means of adapting to local thermal environments. Embryos are more than simply organisms-in-progress, even in early development.

(2) Unlike the case in viviparous species, embryos of oviparous amniotes are exposed to unpredictable thermal variation at a range of temporal and spatial scales. Many ecological factors such as weather conditions, nest structure and location, and the brooding behaviour of parents affect embryonic temperatures in such species. An extensive literature shows that incubation temperatures can have major effects on embryonic development both in reptiles and birds.

(3) Recent work provides a suite of examples whereby embryos are able to avoid, tolerate, or adjust to the unpredictable thermal variation that they encounter during development. A wide range of behavioural, physiological and biochemical mechanisms is involved, at levels from the whole organism to the molecular.

(4) Future research priorities in this emerging field include a better understanding of (i) the thermal challenges and opportunities encountered by embryos in natural nests, (ii) the proximate mechanisms by which embryos deal with their thermal environments, and (iii) the responses of embryos to climate change, and the role of those responses in a species' vulnerability to climate change.

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## VIII. SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

**Table S1.** Data from published literature on hatching success and incubation period at different incubation temperatures in reptiles and birds.

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