biology
letters

Thermally induced torpor in fullterm lizard embryos synchronizes hatching with ambient conditions

Rajkumar Radder and Richard Shine

Biol. Lett. 2006 **2**, 415-416
doi: 10.1098/rsbl.2006.0495

biology
letters

Biol. Lett. (2006) **2**, 415–416
doi:10.1098/rsbl.2006.0495

Thermally induced torpor in fullterm lizard embryos synchronizes hatching with ambient conditions

Rajkumar Radder and Richard Shine*

School of Biological Sciences A08, University of Sydney,
NSW 2006, Australia
**Author for correspondence* (*rics@bio.usyd.edu.au*).

Eggs inside an underground nest have limited access to information about above-ground conditions that might affect the survival of emerging hatchlings. Our measurements of heart rates of embryos inside the intact eggs of montane lizards (*Bassiana duperreyi*, Scincidae) show that low temperatures induce torpor in fullterm embryos, but do not do so during earlier embryogenesis or later, post-hatching. Because above-ground conditions affect soil temperatures, this stage-dependent torpor effectively restricts hatching to periods of high ambient temperatures above ground. Torpor thus can function not only to synchronize activity with suitable environmental conditions during post-hatching life (as reported for many species), but also can occur in embryos, to synchronize hatching with above-ground conditions that facilitate successful emergence from the nest.

Keywords: embryogenesis; metabolic rate; heart rate; reptile

1. INTRODUCTION

Many kinds of animals lay their eggs in underground nests where the embryos are likely to have little access to information about above-ground conditions (such as day versus night, or warm versus cold weather) that could critically affect the offspring's ability to hatch, emerge and disperse. How, then, can hatching time be synchronized with ambient above-ground conditions? The problem is likely to be greatest in habitats where environmental conditions shift unpredictably over short time-scales during the hatching season, in ways that will influence the success of attempts to hatch and emerge from the nest. For example, the eggs of montane scincid lizards (*Bassiana duperreyi*) from southeastern Australia hatch late in the brief alpine summer, when weather is variable and unpredictable (Shine 2002). Hatchlings dig their way out of the nest and disperse as soon as they break out of the eggs (R. Shine 2001, personal observation). Emerging from the nest at night, or during a spell of cold weather, could expose the slow-moving young lizards to danger from predators or lethally low temperatures (Shine 2002). Thus, selection should favour physiological mechanisms that synchronize lizard hatching with suitable (warm) conditions above ground.

How might such a system operate? Two issues are important: what kinds of cues are available inside the nest and what kinds of mechanisms might synchronize hatching with such a cue? The first question is easiest to answer, because heat travels fairly rapidly through the soil profile, and thus nest temperatures accurately mirror ambient thermal conditions (Shine & Elphick 2001). This correlation means that embryos (at least in shallow nests) have more information about above-ground conditions than would be suggested by intuition. Thus, nest temperatures provide a potential cue to conditions in the outside world. However, the second question is more difficult to answer. The ideal mechanism would be one that precluded activity at low temperatures for (and only for) fullterm embryos. Such a facultative (thermally induced) torpor could thus allow both earlier embryos and post-hatching lizards to function effectively (develop, move, etc.) at low temperatures, but prevent the activities necessary for hatching and nest emergence at the critical period when such activity would likely allow nest emergence under unsuitable environmental conditions.

How can we test for the presence of such a mechanism? The hypothesis outlined above predicts that fullterm embryos (but not earlier embryonic stages) will fall into torpor when (and only when) nest temperatures fall low enough to curtail effective locomotion by hatchlings. To test this prediction, we monitored heart rates of lizard embryos and hatchlings as a function of developmental stage and incubation temperature.

2. MATERIAL AND METHODS

We collected female *B. duperreyi* from the Brindabella Range (Shine 2002) in southeastern Australia in November 2005, and kept them in captivity until they oviposited less than two weeks later. Eggs were incubated in individual containers of moist vermiculite (-200 kPa) inside cycling-temperature cabinets set to mimic natural nest conditions (mean 22 °C, diel range 7.5 °C). To measure heart rates, eggs were kept at known temperatures (15, 20, 25 or 30 °C) for 2 hours before being removed from their incubation containers and placed individually in a Buddy infrared heart rate monitor for 2 minutes (Avian Biotech; http://www.avianbiotech.com/buddy.htm). Eggs were tested in random order at each temperature. We used 27 eggs for the first stage, and one less (because an individual was killed each time to assess developmental stage: see below) at each successive stage. Stage of embryogenesis according to the Dufaure & Hubert (1961, as modified by Muthukkaruppan *et al.* 1970 for oviparous species) scale was assessed at each sampling period (15, 30 and 45 days) by dissecting one egg each time: relevant stages were 35, 38 and 42 (hatching occurs at stage 42; eggs began hatching 2 days after we recorded their stage-42 heartbeat rates). To record heart rates of hatchlings, we anaesthetized week-old lizards with gaseous (Fluothane) inhalation, and placed them back inside their empty eggshells.

3. RESULTS

Unsurprisingly, higher temperatures elicited higher heartbeat rates both for embryos and for hatchling lizards (figure 1). The relationship between ambient temperature and the embryo's heart rate was consistent throughout early embryogenesis, and reverted to this same pattern soon after hatching (figure 1). However, pre-hatching embryos temporarily deviated from this relationship (45 days into incubation; repeated-measures ANOVA interaction embryo age versus temperature, $F_{9,270}=9.75$, $p<0.0001$). These fullterm embryos fell into torpor when nest temperatures dropped below 20 °C (figure 1).

Received 5 April 2006
Accepted 21 April 2006

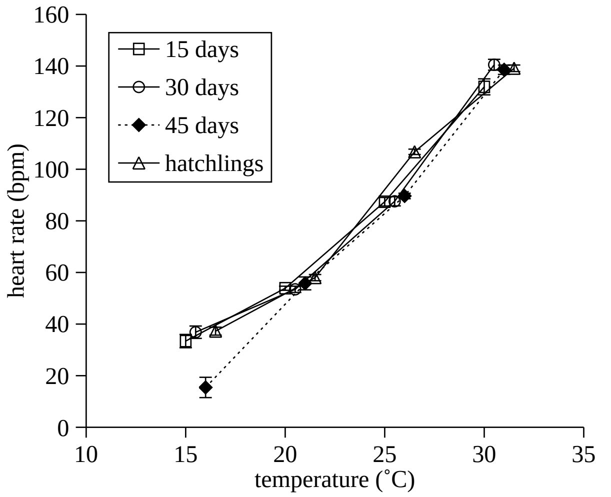

Figure 1. Heart rates of embryonic and hatchling scincid lizards (*Bassiana duperreyi*) monitored inside their eggs at a range of ambient temperatures and at 15-day intervals over the course of embryogenesis and immediately post-hatching. The relationship between heart rate and temperature was very stable throughout development, except for a marked decrease (torpor) in late-stage embryos at low temperatures. This decrease effectively restricts hatching and emergence to times when ambient temperatures exceed 20 °C.

4. DISCUSSION

In the field, *Bassiana* nest temperatures above 20 °C occur only during the afternoon and in good weather (Shine & Elphick 2001). Hence, this stage-dependent torpor would effectively synchronize hatching with above-ground conditions that would facilitate the hatchling's opportunities to emerge from the nest and disperse.

Embryos of many species benefit by matching the hatching event to suitable ambient conditions. A wide variety of environmental cues may act as the trigger, from flooding of terrestrial nests in some anuran amphibians (Seymour *et al.* 1991) and freshwater turtles (Webb 1986) to tidal cycles in some crustaceans (Saigusa 2002). Social cues (from hatching siblings) may also be involved, due to advantages from within-clutch synchrony in offspring emergence times (e.g. birds, Woolf *et al.* 1976; turtles, Thompson 1989; crocodilians, Webb & Manolis 2002). Given the central role of temperature for organismal performance in ectotherms (Grigg *et al.* 2004), synchronizing hatching with warm ambient conditions is likely to be important in a diverse array of vertebrate and invertebrate taxa. To our knowledge, the present study provides the first empirical example of a mechanism that acts in this way.

At first sight, the functional role of torpor in this system appears to be very different from that in the avian and mammalian systems in which it has attracted so much previous study from physiologists. Intriguingly, however, there is a strong parallel between the circumstances that favour torpor in pre-hatching ectotherms versus post-hatching endotherms. Torpor in adult and juvenile endotherms is generally interpreted as an energy-conservation adaptation (Geiser *et al.* 2005), and in many cases essentially functions to synchronize the organism's activity with suitable environmental conditions. That is, torpor results in animals being active only at times and places where their ability to gain energy exceeds the costs of maintaining activity (Grigg *et al.* 2004). Our study suggests that analogous benefits may have favoured the expression of transitory (stage-dependent) torpor during embryonic life also, and in ectotherms as well as endotherms.

We thank M. Elphick and J. Thomas for assistance in the laboratory and the field, M. B. Thompson and F. Seebacher for comments on the manuscript and the Australian Research Council for funding. Our experiments were approved by the University of Sydney Animal Care and Ethics Committee.

Dufaure, J. P. & Hubert, J. 1961 Table de développement du lézard vivipare: *Lacerta (Monotoca) vivipara* Jacquin. *Arch. Anat. Microsc. Morphol. Exp.* **50**, 309–328.

Geiser, F., Law, B. S. & Kortner, G. J. 2005 Daily torpor in relation to photoperiod in a subtropical blossom-bat, *Syconycteris australis* (Megachiroptera). *J. Therm. Biol.* **30**, 574–579. (doi:10.1016/j.jtherbio.2005.08.002)

Grigg, G. C., Beard, L. A. & Augee, M. L. 2004 The evolution of endothermy and its diversity in mammals and birds. *Physiol. Biochem. Zool.* **77**, 982–997. (doi:10.1086/425188)

Muthukkaruppan, V., Kanakambika, P., Manickavel, V. & Veeraraghavan, K. 1970 Analysis of the development of the lizard, *Calotes versicolor*. I. A series of normal stages in the embryonic development. *J. Morphol.* **130**, 479–489. (doi:10.1002/jmor.1051300407)

Saigusa, M. 2002 Hatching controlled by the circatidal clock, and the role of the medulla terminalis in the optic peduncle of the eyestalk, in an estuarine crab *Sesarma haematocheir*. *J. Exp. Biol.* **205**, 3487–3504.

Seymour, R. S., Geiser, F. & Bradford, D. F. 1991 Metabolic cost of development in terrestrial frog eggs (*Pseudophryne bibronii*). *Physiol. Zool.* **64**, 688–696.

Shine, R. 2002 Eggs in autumn: responses to declining incubation temperatures by the eggs of montane lizards. *Biol. J. Linn. Soc.* **76**, 71–77. (doi:10.1046/j.1095-8312.2002.00049.x)

Shine, R. & Elphick, M. J. 2001 The effect of short-term weather fluctuations on temperatures inside lizard nests, and on the phenotypic traits of hatchling lizards. *Biol. J. Linn. Soc.* **72**, 555–565. (doi:10.1006/bijl.2000.0516)

Thompson, M. B. 1989 Patterns of metabolic development in embryonic reptiles. *Respir. Physiol.* **76**, 243–256. (doi:10.1016/0034-5687(89)90101-1)

Webb, G. J. W. 1986 Nests, eggs and embryonic development of *Carettochelys insculpta* (Chelonia: Carettochelyidae) from northern Australia. *J. Zool. (Lond.)* **208**, 521–550.

Webb, G. & Manolis, C. 2002 *Australian crocodiles: a natural history*. Sydney, Australia: Reed New Holland.

Woolf, N. K., Bixby, J. L. & Capranica, R. R. 1976 Prenatal experience and avian development: brief auditory stimulation accelerates the hatching of Japanese quail. *Science* **194**, 959–960.