Embryos in the Fast Lane: High-Temperature Heart Rates of Turtles Decline After Hatching

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Abstract
In ectotherms such as turtles, the relationship between cardiovascular function and temperature may be subject to different selective pressures in different life-history stages. Because embryos benefit by developing as rapidly as possible, and can “afford” to expend energy to do so (because they have access to the yolk for nutrition), they benefit from rapid heart (and thus, developmental) rates. In contrast, hatchlings do not have a guaranteed food supply, and maximal growth rates may not enhance fitness—and so, we might expect a lower heart rate, especially at high temperatures where metabolic costs are greatest. Our data on two species of emydid turtles, Chrysemys picta, and Graptemys pseudogeographica kohnii, support these predictions. Heart rates of embryos and hatchlings were similar at low temperatures, but heart rates at higher temperatures were much greater before than after hatching.

Introduction
Hatching (or birth, in viviparous species) entails a profound shift in the physiological functioning of any amniote vertebrate. The offspring must make a rapid transition between the embryonic dependence on extra-embryonic membranes for respiration and excretion, to the free-living stage of reliance on organ systems such as lungs, kidneys, sense organs, skeletal muscles, and the like. Unsurprisingly, then, hatching is accompanied by major shifts in the organism’s physiology [1]: not only the acquisition of some functions and the loss of others, but also a modification of the norms of reaction that link organismal function to variable external conditions. One such relationship is the rate at which metabolic processes increase with an increase in body temperature. Although a positive relationship between these two variables is predicted by simple physics and chemistry [2,3], the exact form of the relationship is flexible, and can be modified by developmentally plastic responses such as acclimation [4].

How might we expect the relationship between temperature and metabolic rate (and correlates of metabolic rate, such as developmental rate and cardiovascular functioning [5]) to be affected by the life-history transition from embryo to hatching? We suggest that embryonic life should favour a steep reaction norm, whereby metabolic rate increases sharply at higher temperatures. This prediction is based on the embryo benefiting from (a) accelerated developmental rate, because earlier hatching typically enhances offspring fitness [6,7]; and (b) the assured food supply (in the yolk) to fuel development through to hatching [8]. Both of these factors shift abruptly when the egg hatches. Hatchlings face uncertain levels of food availability, and their fitness often may be enhanced by less-than-maximal rates of growth [9]. Thus, we might expect natural selection to fine-tune thermal norms of reaction, such that higher temperatures induce less of an increase in hatchling metabolic (and thus, heart beat) rates in free-living animals than was the case prior to hatching. In the current paper, we test this prediction with data on heart rates relative to temperature in embryos and hatchlings of two species of emydid turtles.

Materials and Methods
Study Species
Chrysemys picta, and Graptemys pseudogeographica kohnii are two North American freshwater turtles belonging to the family Emydidae. Both species live in ponds, lakes, marshes, and slow-moving rivers [10]. Female C. picta lay 4 to 15 oval, soft shelled eggs in shallow nests with mean temperatures from 24 to 28°C [11], and the hatchlings typically spend the first winter of their life in the nests [10]. Both C. picta and G. pseudogeographica kohnii can elevate their body temperatures above water temperature by basking [12,13]. Shell temperatures of C. picta can vary from 15 to 35°C during the active season from April to October [12].

Egg Incubation and Measurement of Heart Rate
In May, 2009, we collected freshly-laid eggs of two turtle species, C. picta, and G. pseudogeographica kohnii, from a private farm in Hankou, Huanan province of China. All eggs were weighed (±0.001 g), and individually incubated at 28°C in 120 ml glass jars filled with moist vermiculite (~200 KPa). Heart rates were measured using an infrared heart rate monitor (Buddy system; Avian Biotech; for detailed methods see [5]). High positive correlations between heart rates and metabolic rates both in...
embryos [14,15] and post-hatching individuals [16] mean that heart rates can serve as an index of metabolic rate. The heart rates of embryos were measured twice during incubation, at approximately 25% and 75% through the total incubation period, and the heart rates of hatchlings were measured on the first and tenth day after hatching. To ensure test conditions were similar between embryos and hatchlings, we placed hatchlings inside perforated egg-shaped plastic balls to measure their heart rates. Eggs and hatchlings were allowed to acclimate to test temperatures for two hours (inside incubators set at 20, 25, 30, or 33.5°C), and were then placed individually on the monitor to record heart rate (which generally required less than a minute). The order of exposure of each egg or hatchling to test temperatures was random. Heart rates were repeatedly measured on the same samples of C. picta (n = 16), and G. pseudogeographica kohnii (n = 15) from eggs to hatchlings. Egg collection and experimental procedures were approved by the Animal Care and Ethics Committee of Hangzhou Normal University and were conducted in accordance with the NIH Guide for the Principles of Animal Care.

Repeated measures ANOVAs with species and life-history stage as factors and test temperatures as the repeated variable were conducted to test for ontogenetic shifts and between-species differences in heart rate at different environmental temperatures. One-way ANOVA and Tukey’s post-hoc tests were then used to compare heart rate among different life-history stages at each test temperature of each species.

**Results**

Eggs of both species (C. picta and G. pseudogeographica kohnii) hatched after mean incubation periods of 57 and 66 days, respectively. In an analysis including data for both embryos and hatchlings of the two species of turtles, heart rate was significantly affected by test temperature ($F_{3,341} = 813.9$, $P<0.00001$), and changed ontogenetically ($F_{3,116} = 20.8$, $P<0.00001$), but did not differ significantly between species ($F_{1,116} = 0.12$, $P = 0.73$). Heart rates did not differ between successive developmental stages either in embryos or hatchlings, but differed between embryos and hatchlings, with lower heart rates at high temperatures for hatchlings than for embryos (Fig. 1). $Q_{10}$ values of heart rate varied from 1.5 to 2.6 in embryos and from 1.4 to 1.9 in hatchlings depending on temperatures, and differed between embryos and hatchlings, with higher $Q_{10}$ values in embryos (Table 1).

**Discussion**

The two species of turtles that we studied exhibited similar ontogenetic shifts in heart rate. At the lowest temperature we tested (20°C), heart rates were unchanged from the embryo through to the hatchling stage. At higher temperatures, however, hatchlings exhibited slower heart rates than did embryos (by about 30% at 30°C; see Fig. 1). Because we sampled at only four stages during ontogeny, we cannot identify the exact timing of this developmental shift except to say that it occurred late in embryogenesis (i.e., in the final 25% of the incubation period). Oxygen consumption (and hence energy expenditure) decreases during the last few weeks of incubation in turtles due to rapid decrease of the rate of new tissue synthesis [17]. Heart rates relative to temperature were similar in early and late-stage embryos in our study, suggesting that a decrease might occur close to the time of hatching, and be maintained in post-hatching life. The mechanisms responsible for the ontogenetic shift in heart rates are unknown, but may relate to maturation of cardiovascular control mechanisms in late-term embryos, including both hormonal and nervous regulatory systems [18].

Limited data on other species suggest considerable diversity in the ontogenetic progression of reaction norms linking heart rates to temperature. As in the two turtle species that we studied, embryos of European pond turtles (Emys orbicularis) and common snapping turtles (Chelydra serpentina) reduce heart rates shortly before hatching [19,20] as well as after hatching [21]. The low post-hatching heart rates appear to be maintained through into adult life; for example, heart rates of adult Trachemys scripta, Terrapene ornate and Gopherus agassizi are only about 50, 57 and 30 beats/min at 30°C [22,23,24]. Thus, shifts in reaction norms linking heart rates to temperature appear to be widespread in turtles. Squamate reptiles show a different pattern, with little or no ontogenetic shift in heart rates from embryo to hatchling stages (e.g., the skink Bassiana dupreysi - [25]). $Q_{10}$ coefficients of eastern fence lizards (Sceloporus undulatus) are similar between embryos and hatchlings both for metabolic rate (2.1 vs 2.4) [26,27] and heart rate (2.4 vs 2.3) [28]. In contrast, most bird embryos exhibit an increase in heart rates around the time of hatching [29].

Why do turtles show a decrease in heart rates at hatching, squamates show no shift, and birds show an increase? The data on
birds are explained by the development of endothermic metabolism, and hence an elevation in metabolic rate [29]. Our data on turtles are consistent with the hypothesis outlined in the Introduction to this paper. That is, shifts in the benefits versus costs of high metabolic rates (and thus, heart rates) between the egg stage and the free-living juvenile may impose selection for adjustment of cardiovascular function. Metabolic rates at low temperatures probably have little impact on fitness in either life-history stage, because energy-expenditure costs are low and little embryogenesis is accomplished at such temperatures [24,30]. In contrast, metabolic rates at high temperatures likely are under more intense selection for fine-tuning relative to the organism’s life-history stage, because both the costs (in energy expenditure) and potential benefits to the embryo but not the hatching (i.e., rapid embryogenesis) are high. Moreover, in hatching C. picta that overwinter in shallow nests, low heart rates at higher temperatures could also reflect a preparation for low-temperature hibernation, as reported in adult turtles [31].

If turtles benefit by reducing heart rates at hatching, why don’t lizards show the same pattern? Annual survival rates (and thus, longevities) average much higher in turtles than in squamates, in both juvenile and adult life-history stages [32]. As a result, short-lived lizards may adopt “riskier” life-history tactics, expending energy at high rates despite the uncertainty about future food availability [9]. The wide range in adult survival rates in squamates [32] provides abundant opportunity to test this explanation, because it predicts decreased heart rates at hatching in long-lived but not short-lived species. Unfortunately, we have so little data that generalizations may be premature.

All oviparous reptiles produce eggs with sufficient yolk to support embryogenesis to the hatching stage; indeed, a portion of that initially-allocated energy (residual yolk) often is used for maintenance of hatchlings during early post-hatching life [8,33]. Because embryos have sufficient energy to complete development, and are likely to benefit from early hatching [6,7], selection should favour adaptations that result in more rapid development within the egg. Given the embryo’s inability to thermoregulate behaviourally, increasing metabolic (and thus, developmental) rate at high temperature is probably the most important mechanism available to accelerate embryonic development and growth. Retaining such high metabolic rates post-hatching may enhance offspring fitness in some species (especially, short-lived taxa or those encountering abundant food), but for many post-hatching reptiles food availability is limited, and may vary unpredictably. Under conditions of low food supply, many reptiles select lower body temperatures, thereby reducing maintenance metabolic costs [34,35]. A reduction in the slope of the reaction norm linking metabolic rate to temperature (Fig. 1) would result in a similar energy saving. Lifespan is correlated with a fixed [species-specific] number of heart beats in birds and mammals, with lower heart rates in species showing higher longevities [10]. The low heart rate of turtle hatchlings thus may be related to the higher longevities of these species.

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Author Contributions
Conceived and designed the experiments: WGD. Performed the experiments: WGD BZ. Analyzed the data: WGD. Wrote the paper: WGD RS.

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Table 1. Q10 values of heart rate in different life-history stages in two species of turtles, Chrysemys picta and Graptemys pseudogeographica kohnii.

|                | 20–25 C | 25–30 C | 30–33.5 C |
|----------------|---------|---------|-----------|
| Chrysemys picta|         |         |           |
| Embryo, Day 15 | 2.20    | 1.83    | 1.79      |
| Embryo, Day 40 | 2.19    | 1.74    | 1.85      |
| Hatchling, Day 1| 1.87   | 1.40    | 1.42      |
| Hatchling, Day 10| 1.55  | 1.49    | 1.72      |
| Graptemys pseudogeographica kohnii| | | |
| Embryo, Day 15 | 2.38    | 1.63    | 1.51      |
| Embryo, Day 40 | 2.64    | 1.78    | 1.62      |
| Hatchling, Day 1| 1.78   | 1.58    | 1.35      |
| Hatchling, Day 10| 1.39  | 1.84    | 1.35      |
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