An ontogenetic shift in the response of heart rates to temperature in the developing snapping turtle (*Chelydra serpentina*)

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Received 24 January 1999; received in revised form 22 August 1999; accepted 20 September 1999

Abstract

The effect of acute changes in temperature on heart rates was investigated for the first time in a developing reptile. Heart rates were determined early and late in incubation in snapping turtle (*Chelydra serpentina*) eggs. Late in incubation heart rates at any given temperature were lower than those observed early in incubation. The results of temperature switching experiments late in incubation were consistent with thermal acclimation.

Keywords: Heart rate; Reptile; Egg; Temperature; Development; Turtle; *Chelydra*; Ontogeny; Acclimation

1. Introduction

Incubation conditions affect developing reptiles (Deeming and Ferguson, 1991) and hatchling phenotype (Van Damme et al., 1992; Shine et al., 1997; Packard et al., 1999). Among the variables studied, temperature exerts the most profound effects. In developing embryos incubation period, growth rates, oxygen consumption rates, acid-base balance and sex are altered by changes in temperature (Birchard et al., 1990; Deeming and Ferguson, 1991; Booth, 1998). Examination of some physiological and growth measures in snapping turtles developing at two different temperatures have suggested that the physiological response to temperature may not be constant over the course of development in some reptiles (Birchard and Reiber, 1995, 1996; Yntema, 1978). Measurements of growth and oxygen consumption early in development (first half of the incubation period) do not indicate compensation to chronic temperature changes. However, late in incubation (last half of incubation) these physiological rates were similar at the two different temperatures (Birchard and Reiber, 1995). Subsequent examination of heart rates over the course of the development in the snapping turtle were consistent with an ontogenetic change in the response to temperature (Birchard and Reiber, 1996). These data suggest that the capacity for thermal acclimation, and thus the affect of temperature, changes with development in this species.

Most studies of reptile eggs including those on snapping turtles have examined the affect of constant temperatures on physiological processes. In natural nests reptile eggs are subjected to temperature variations both daily and over the course of incubation (Packard...
et al., 1985; Thompson et al., 1996). The physiological responses to acute temperature changes and short-term acclimation have not been well studied in developing reptiles. Examination of acute responses at various times during development should also be useful in diagnosing ontogenetic changes in physiological systems. Further, because the magnitude of short-term changes in nest temperature observed in the wild correspond with the temperature differences affecting embryos and hatchlings in constant temperature experiments, it seems likely that measurements of physiological responses within this time frame will prove useful in understanding the physiology of embryos in natural nests. In this study, the response of heart rates of developing snapping turtles to acute temperature changes with and without short-term temperature acclimation were examined. Because evidence exists for an ontogenetic change in the response to temperature in this species, measurements were made both early and late in incubation.

2. Materials and methods

Eggs from five clutches of snapping turtles (*Chelydra serpentina*) were collected on the Valentine National Wildlife Refuge, Cherry County, Nebraska, USA, during June 1997. Eggs were packed in damp vermiculite in a plastic cooler and transported to George Mason University. Eggs from snapping turtle clutches were divided into two groups and incubated at either 24°C or 29°C. Late in incubation some snapping turtle eggs were moved from their initial temperature treatment to the other for three days (24°C eggs on day 61 of incubation) and five days (29°C eggs on day 46 of incubation) to study the response to short-term acclimation.

Heart rates were determined by measuring impedance changes between two Teflon-coated stainless steel electrodes (180 μm in diameter) implanted through the shell. The procedures followed were similar to those described previously, with the following modifications (Birchard and Reiber, 1996). Electrodes were implanted the day before experiments were to be carried out. Following implantation of the electrodes, eggs were placed in a small chamber on moist vermiculite. This chamber was mostly submerged in a circulating water bath. The initial temperature of the water bath was the same as the temperature of the incubator from which the eggs had been obtained. The temperature near the egg surface was monitored continuously with a Sensortech TH-6D thermocouple thermometer. The day following the implantation of electrodes impedance changes from each egg were determined and then the temperature of the chamber was raised or lowered 3–4°C. Once the thermometer showed that the desired chamber temperature had been reached, eggs were equilibrated for at least an hour. Heart rates were then determined and the process of changing the temperature repeated. Experiments lasted about 12 h. At the end of the experiment the temperature of the water bath was returned to the initial temperature. Heart rates were determined the following morning to check for experimental effects. In snapping turtle eggs incubated at 29°C heart rates were determined on days 24 and 50. Heart rates were measured on days 31 and 65 in 24°C incubated eggs. Sampling times were chosen on the basis of previous data (Birchard and Reiber, 1996) to produce similar developmental stages and masses of developing turtles from the 24°C and 29°C incubated eggs. Heart rates were measured over a temperature range of 4.4°C to 30.7°C. To ensure comparisons between treatments were made over a similar temperature range, the analyses presented here were restricted to the temperature range 9.0–30.7°C. Measurements were obtained consistently from most eggs. However, because of embryo movement it was not always possible to obtain a signal at a given temperature on every egg. The number of eggs in each treatment group and average number of eggs from which measurements were obtained at each temperature are given in Table 1.

Data are presented as the mean ± 1 SD. Least squares regression followed by an analysis of variance

### Table 1

Regression equation coefficients and statistics for Arrhenius plots of heart rates in Figs. 1 and 2. Equations are of the form $\log(H-R) = \text{intercept} + \text{slope}(1/T \times 1000)$. $n$ is the number of eggs from which measurements were obtained, SE is the standard error. Mean samples is the average number of eggs from which measurements were obtained at each temperature.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$n$</th>
<th>Mean samples</th>
<th>Slope (SE)</th>
<th>Intercept (SE)</th>
<th>$r^2$</th>
<th>$Q_{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>29°C early incubation</td>
<td>5</td>
<td>3.7</td>
<td>-3.950 (0.225)</td>
<td>15.014 (0.761)</td>
<td>0.94</td>
<td>2.48</td>
</tr>
<tr>
<td>24°C early incubation</td>
<td>5</td>
<td>4.6</td>
<td>-3.508 (0.099)</td>
<td>13.555 (0.332)</td>
<td>0.98</td>
<td>2.24</td>
</tr>
<tr>
<td>29°C late incubation</td>
<td>4</td>
<td>4</td>
<td>-3.127 (0.221)</td>
<td>12.076 (0.752)</td>
<td>0.92</td>
<td>2.05</td>
</tr>
<tr>
<td>24°C late acclimated to 29°C</td>
<td>4</td>
<td>3.4</td>
<td>-2.850 (0.211)</td>
<td>11.163 (0.717)</td>
<td>0.92</td>
<td>1.93</td>
</tr>
<tr>
<td>24°C late incubation</td>
<td>5</td>
<td>4.6</td>
<td>-2.780 (0.256)</td>
<td>11.005 (0.861)</td>
<td>0.80</td>
<td>1.90</td>
</tr>
<tr>
<td>29°C late acclimated to 24°C</td>
<td>3</td>
<td>3</td>
<td>-2.903 (0.315)</td>
<td>11.404 (1.063)</td>
<td>0.82</td>
<td>1.95</td>
</tr>
</tbody>
</table>
was applied to heart rate data presented as an Arrhenius plot. Differences between treatments were evaluated by analysis of covariance when appropriate. A $P$ value of less than 0.05 was considered significant. All statistical analyses were done with the NCSS statistical package.

3. Results

The mean control heart rate for eggs incubated at 24°C early and late in incubation were 53.4 ± 4.5 and 47.5 ± 5.6 beats/min$^{-1}$ respectively. In eggs incubated at 29°C the mean control heart rate early in incubation was 98.4 ± 1.7 and late in incubation 52.0 ± 5.8 beats min$^{-1}$. The Arrhenius plots of heart rates (Figs. 1 and 2) appear to be linear over the temperature ranges studied. The heavy concentration of sampling in the central part of the plot is the result of: (1) making changes in temperature of no more than 4°C at one time and doing measurements both above and below the initial (incubation) temperature, thus the experiment temperatures necessarily passed near the initial point at least once; and (2) a final control measurement. Initial and final heart rates within any experimental run for embryos did not show a different pattern. The $Q_{10}$ for snapping turtles varied with the day of incubation and temperature treatment (Table 1).

The hearts of snapping turtle embryos incubated at 29°C had a slightly higher sensitivity to the temperature than embryos from eggs incubated at 24°C. A significant change in the response of heart rates to temperature occurred with incubation. Heart rates of snapping turtles early in incubation appear to be more sensitive to changes in temperature than heart rates late in incubation (Fig. 2, Table 1). Further, the heart rates were significantly lower later in incubation within the range of temperatures where normal development occurs (Fig. 1, Table 1).
Examination of the data from eggs incubated at 24°C and 29°C late in incubation indicates that the acute response of heart rates to temperature show a similar sensitivity to temperature (Table 1). However, the heart rates of developing turtles incubated at 29°C are significantly lower than those incubated at 24°C at any given temperature (Fig. 2, Table 1). The five- and three-day exposures of fetal snapping turtles to the alternate experimental temperature changed the response of heart rates to match those chronically incubated at that temperature and significantly different from those at their original incubation temperature (Fig. 2 and Table 1).

4. Discussion

Early in incubation control heart rates of eggs incubated at 29°C were significantly higher than those of eggs incubated at 24°C. However, late in incubation the heart rates of eggs incubated at 29°C decreased and were not different from those incubated at 24°C. These heart rates correspond well both in magnitude and temporal pattern with those measured previously in developing snapping turtles (Birchard and Reiber, 1996).

This the first report on the response of heart rates to acute changes in temperature in developing reptiles. The acute responses to temperature observed here are similar to those observed in developing fish, amphibians and birds (Mirkovic and Rombough, 1998; Rome et al., 1992; Tazawa and Nakagawa, 1985). The Arrhenius plots and $Q_{10}$s between two and three for snapping turtles are also typical of those found in adult reptiles (Stinner, 1987; White, 1976). The concordance of the responses during development with those observed in adults of this species is difficult to assess because of the lack of data and/or the different temperature ranges over which measurements were made.

Previous work indicated that the responses to temperature in developing snapping turtles changed over the course of development (Birchard and Reiber, 1995, 1996; Yntema, 1978). On the basis of these earlier studies it was hypothesized that temperature acclimation occurred during the last 40–50% of the incubation period. Heart rate data support this hypothesis. The higher heart rates previously measured in eggs incubated at 29°C during the first half of incubation (Birchard and Reiber, 1996) appear to be almost solely due to temperature (Fig. 1). There is little indication of any acclimatory response over the range of incubation temperatures used during the early development in this species. However, late in incubation, the response of heart rates to temperature is significantly different from that observed early in incubation (Figs. 1 and 2, Table 1). Further, examination of Arrhenius plots for eggs incubated at 29°C and 24°C suggests complete acclimation occurred. That acclimation takes place is confirmed by the heart rate responses of eggs swapped to the other temperature for a three- or five-day period. The swapped eggs had a response to temperature indistinguishable from eggs which were chronically incubated at the same temperature. Further, the short time necessary for completion of the acclimatory process would indicate that this response takes place during natural incubation.

At this time evidence for thermal acclimation in developing reptiles exists only during the last half of the incubation period (Birchard and Reiber, 1995, 1996; Booth, 1998). That is, there is an ontogenetic change in the physiological response observed. In a temperate zone reptile nesting at higher latitudes thermal acclimation would appear to be advantageous and its absence early in incubation seems puzzling. It is possible that this type of regulatory response requires well developed and coordinated nervous and endocrine systems. The morphological and physiological bases for such responses may be absent in early embryos. Alternatively, it is also possible that the implementation of thermal acclimation during the first half of incubation is constrained in some species. The snapping turtle has temperature-dependent sex determination (Yntema, 1976). Because thermal acclimation acts to decrease the effects of temperature on physiological processes the reactions controlling sex determination might be disrupted if thermal acclimation took place during the critical period. For example, a decreased sensitivity to temperature could widen the pivotal range of temperatures. A wider range of pivotal temperatures might result in an increase of phenotypes where gonadal and behavioral sex are not congruent decreasing fitness (Gutzke and Crews, 1988; Flores et al., 1994). Thus, it may be the ontogenetic change in the response to temperature observed in snapping turtles represents a developmental compromise between sex determining processes and the advantages of thermal acclimation on development (e.g. timely hatching and/or the production of hatchlings having greater fitness) (Shine et al., 1997; Packard et al., 1999).

Acknowledgements

I thank G. Packard, M. Packard and K. Miller for their assistance in collecting turtle eggs. Collecting was authorized under the Nebraska Game and Parks Scientific Permit No. 16. I thank R.R. Huber and L. McDaniel for extending permission to collect on the Valentine National Wildlife Refuge.
References


