Noninvasive heart rate detection using a digital egg monitoring system in *Norops sagrei* embryos

Cassie Guiffre, Austin Hulbert, Dani Douglas
Mentors: Tim Mitchell, Josh Hall, and Dan Warner
Dept. of Biological Sciences, Auburn University
Warner Laboratory of Evolutionary Ecology

Background

- The Buddy® system is a digital egg monitoring system that transmits infrared light to noninvasively detect embryonic heart rates. It was designed to detect the heart rate of bird embryos. Since its conception, it has been useful in detecting heart rates of non-avian reptile embryos. This technology has been used in species that produce large eggs to address the following questions:
  - Are there fixed heart beats in reptilian embryos before hatching?[1]
  - Is there a change in heart rate from embryo to juvenile stage?[2]
  - How do heart rates change through development?[3]
- Heart rates are an important physiological trait. Changes in heart rate can provide an idea of the ways in which differing incubation conditions affect an embryo.
- *Norops sagrei*, the brown anole, is an ideal study species because they are easy to breed in the lab and highly fecund. Due to their small size, the feasibility of measuring their heart rates with the Buddy® system is unknown.

Research Questions
1) Is the Buddy® system an appropriate tool to measure heart rates of *Norops sagrei*?
2) How do heart rates change under fluctuating temperatures throughout embryonic development of *Norops sagrei*?

Time	Temperature (°C)
0700	28.4
1100	30.9
1500	32.0
1800	31.1

Table 1. Incubation temperatures.

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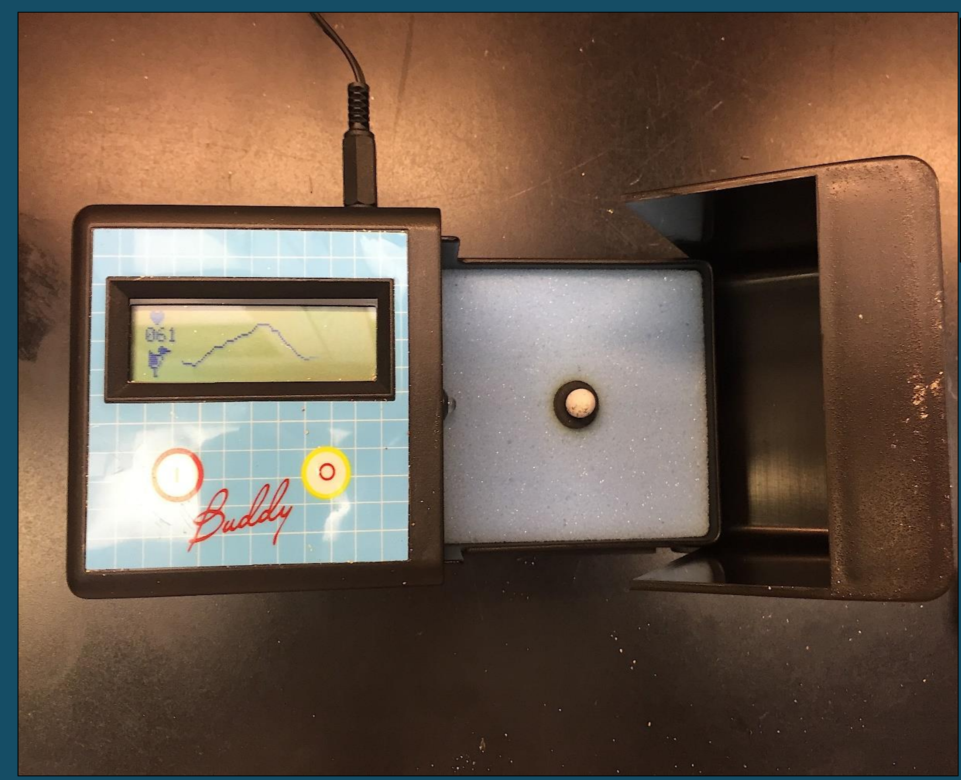

Figure 1. Avitronics Buddy® digital egg monitor.

Figure 2. *N. sagrei* eggs at period 3 (left) and period 1(right).

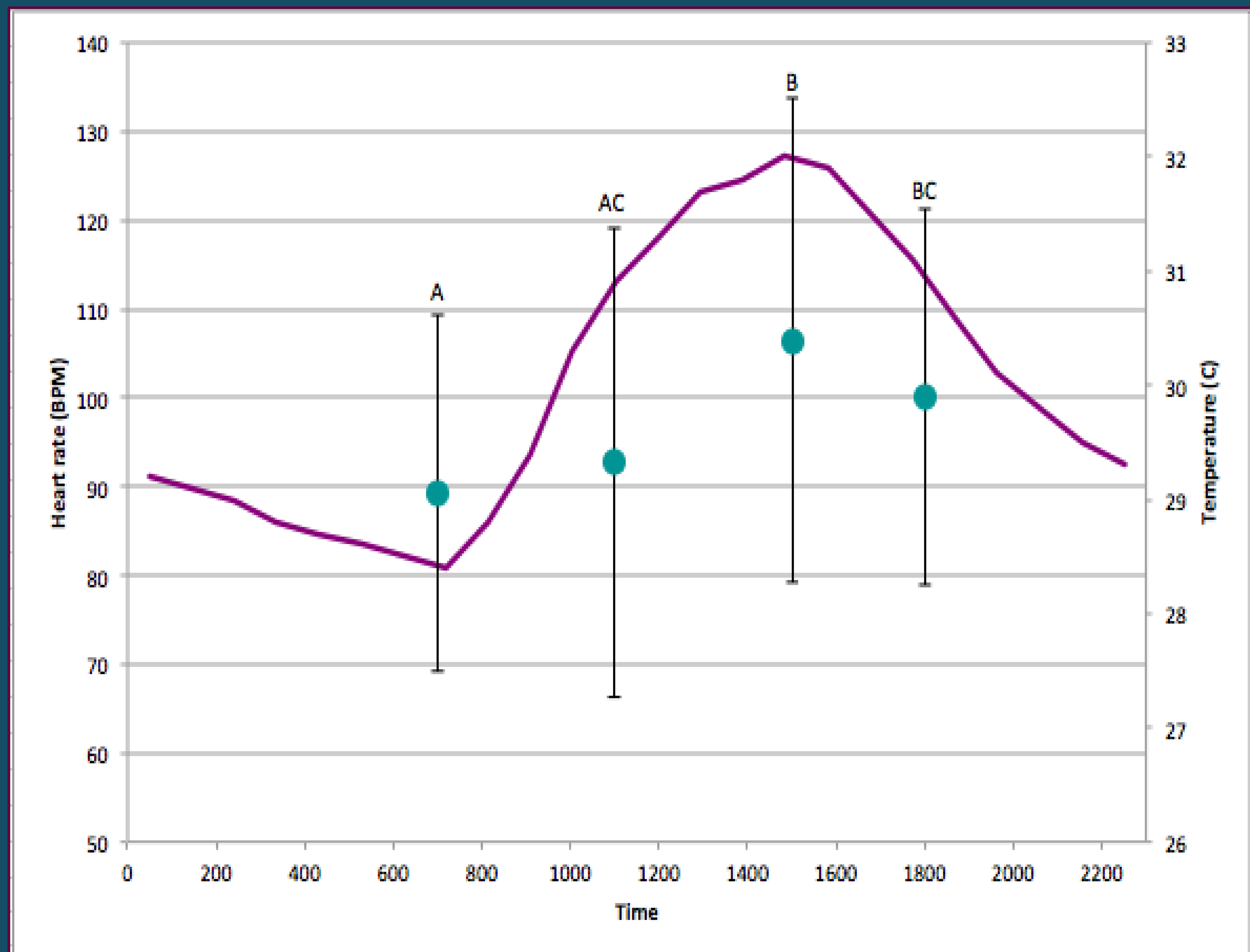

Figure 3. Average embryonic heart rate at fluctuating temperatures mimicking natural, daily temperature regimes. Error bars represent the standard deviation. Differing letters denote a significance at $p<0.05$.

Methods Summary

- To acquire eggs, we bred *N. sagrei* from a captive colony. Incubation temperatures fluctuated, mimicking natural, daily temperature regimes.
- We categorized eggs into four periods – period 1 (1 to 6 days old) period 2 (7 to 13 days old), period 3 (14 to 19 days old) and period 4 (20 days or older).
- We measured heart rates of the eggs using the Buddy® system at four different times with varying temperatures. Number of repositions and duration were recorded. After heart rate was obtained, mass was recorded.
- We measured heart rates of 112 eggs that had been randomly allocated to a time of day for single use. We ensured each time contained relatively even amounts of eggs from each age class.

Results & Conclusions

- There is a significant increase in heart rate with temperature (Figure 3).
- Evidence suggests that age has no effect on heart rate of the embryo ($F_{3, 197}$=1.49, P=0.21).
- We are repeating this experiment using a repeated measures design to compare the utility of these different study designs.
- The Buddy® system is effective in measuring heart rate of *N. sagrei* embryos. We plan to conduct further research and refine our methods to ensure optimal effectiveness.
- In the future, we plan to take the internal temperature on a number of eggs during heart rate detection to determine if there is a significant change in temperature from its removal from the incubator to when the heart rate is detected. A significant change in temperature may physiologically affect the embryo, thereby altering the heart rate.

Literature Cited:
1)Du et al. Determinants of incubation period: do reptilian embryos hatch after a fixed total number of heart beats. 2009. The Journal of Experimental Biology, 212: 1302-1306. 2) Aubret. Heart rates increase after hatching in two species of Natricine snakes. 2013. Scientific Reports, 3: 3384. 3)Sartori et al. An appraisal of the use of an infrared digital monitoring system for long-term measurement of heart rate in reptilian embryos. 2015. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 188: 17-21.