

## EFFECTS OF TEMPERATURE ON THE DEVELOPMENT OF THE WOOD FROG, *RANA SYLVATICA*

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### ABSTRACT

Eggs of the wood frog *Rana sylvatica* were collected from a temporary pond and placed into three controlled chambers with temperatures of 9°C, 21°C and 26°C. As a control, eggs were also placed in an outside container with an average temperature of 11.2°C. The eggs remained in the four containers for 21 days. Their development was regularly monitored and their average size and hatching time recorded. Significant differences were found in the growth rates of the frogs placed at the different temperatures when compared to the control, with the frogs placed in the cooler temperature having a slower growth rate, and the frogs placed in the warmer temperatures having a faster growth rate than the control. There were also noticeable differences in hatching time between the four temperatures. These data suggest that temperature does play a role in the development of the wood frog.

*Keywords: development, global warming, Rana sylvatica, temperature, wood frog*

### INTRODUCTION

Since the 19<sup>th</sup> century when the Industrial Revolution began in the United States, global warming has been a growing concern. Global warming is defined as “the world wide trend towards warmer and warmer temperatures” (Mayer 248). Global warming is believed to be caused primarily by increased levels of greenhouse gases such as carbon dioxide, ozone and chlorofluorocarbons in the atmosphere. Carbon dioxide is the most prevalent greenhouse gas and so its prevention is of the highest concern. A rise in CO<sub>2</sub> levels is caused primarily by the burning of fossil fuels, which is one reason why alternative energy is such a crucial necessity (Mayer, 2001).

The prevention of global warming is being investigated by over 25,000 scientists worldwide. However, recent temperature analyses have shown a drastic increase in temperature over the last 100 years (Figure 1). This rise in temperature poses serious environmental threats to many animals

and plants, and could have drastic effects on their development and life styles.

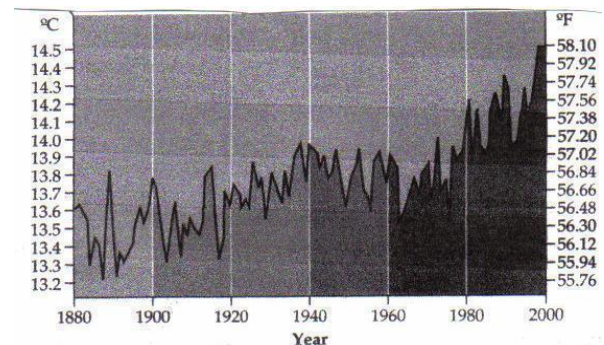


Figure 1. Average global temperature between 1880 and 1998 (Mayer 254)

Ectothermic mpmibians are particularly affected by global warming and extreme temperatures

since their body temperature is not internally regulated but changes with the environment. Additionally, amphibians depend on ephemeral ponds for breeding. Due to their small size, these ponds are easily affected by changes in the climate. A rise in temperature can cause them to become extremely warm, which can affect their ability to sustain life (Halliday, 2002).

To test the effects of temperature on amphibian development we chose to conduct an experiment that tested the effects of different temperatures on the development of the wood frog. Wood frogs are common in Pennsylvania and are distributed throughout much of the northern United States, as well as Canada and Alaska. They are known to breed in both temporary and permanent bodies of water, but prefer to use temporary ponds since there are fewer predators present. Due to their reliance on these ephemeral ponds the wood frog is extremely susceptible to temperature changes. This can be detrimental to its existence since optimal growth has been found to occur at a temperature of approximately 22°C and temperature extremes have been found to be deadly (Censky, 2001). Therefore, we hypothesized that over the course of our twenty-one day study, the frogs placed in a container with a water temperature close to 21°C will have a higher growth rate and earlier hatching time than the eggs placed in the two extremes.

## FIELD SITE

On March 19, 2004, *Rana sylvatica* egg samples were obtained from a puddle along the power lines located near the Raystown Field Station in Huntingdon, Pa. The water in the puddle was approximately six inches deep, muddy and stagnant. There was very little vegetation present, only patchy areas of grass and algae. There were multiple puddles in the area, but this one was the only puddle that contained eggs.

## METHODS AND MATERIALS

The eggs were extracted from the puddle using a bucket, and transported back to Juniata College where they were placed in three temperature-control chambers containing approximately 2L of water, which was obtained from Warm Springs, a neutral fresh water spring in Huntingdon Pa. The control chambers were set at 9°C, 21°C and 26°C. There was also a container filled with 2L of water left outside to simulate the frog's natural environment, from which we obtained our expected values for growth rate and

hatching time. The eggs were then separated into approximately four equal masses by use of a spatula and placed into the four containers. Two liters of water from the puddle in which they were found was also poured into the containers.

The eggs were checked for average size, and the date when the first eggs from each group began to hatch was recorded. The water was checked for the dissolved oxygen level and temperature. Any other observations were also noted. Data were collected on a daily basis except for on the weekends. When the dissolved oxygen level was low, fresh water was added and a bubbler was placed in the container. Once the eggs hatched they were fed algae pellets as needed, and their activity was recorded. A t-test was then conducted comparing the slopes (regression coefficients) of body size vs. time at the different temperatures (Zar, 1984).

## RESULTS

Among the different temperatures there was a noticeable difference in the day of hatching (Table 1). The t-test showed that the growth rate of the frogs placed in a temperature of 9°C was significantly less than the growth rate of the frogs placed in the control, and that the growth rate of the frogs placed at a temperature of 21°C was significantly greater than that of the control frogs (Table 2). The tadpoles' growth rates are shown in Figure 2, and a regression analysis of their development in relation to temperature is shown in Figure 3, with the slope values recorded in Table 3. It was also found that as the temperature of the water increased, the average dissolved oxygen level of the water decreased (Table 4).

Table 1. Hatching time of *Rana sylvatica* at different temperatures

Temperature	9°C	21°C	26°C	Control (outside)
Day Hatched	Day 15	Day 13	Day 4	Day 11

Table 2. T-tests comparing the mean slopes of the frogs placed at 9°C and 21°C in relation to the slope of the control

Temperature	9°C	21°C
t-value	- 3.45	8.086
P-value	<0.001	<0.001

**DISCUSSION**

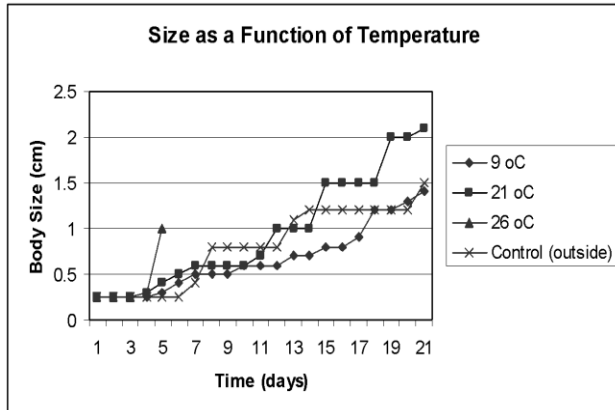


Figure 2. Tadpole growth at different temperatures

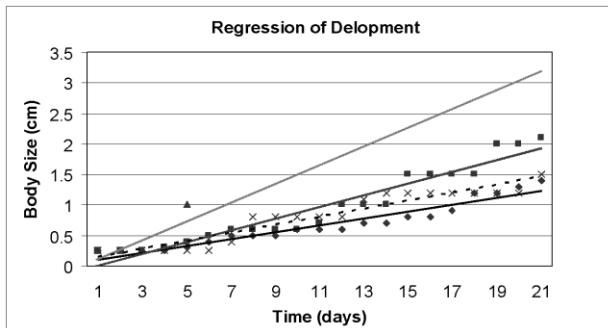


Figure 3. Regression analysis of *Rana sylvatica* development in relation to temperature

Table 3. Slope calculations from the regression chart

Temperature	9°C	21°C	26°C	Control (outside)
Slope	0.056	0.096	0.155	0.066

Table 4. Mean concentrations of dissolved oxygen at the different temperatures

Temperature	9°C	21°C	26°C	Control (outside)
Mean dissolved O <sub>2</sub> level	9.78	7.52	4.20	10.1

As expected from our hypothesis, temperature does play a role in the development of the wood frog. The frogs placed at 9°C had a growth rate that was significantly lower than that of our control group, which had an average temperature of 11.2 °C, and the frogs placed at a temperature of 21°C had a growth rate significantly higher than that of the control group. The growth rate of the frogs placed at a temperature of 26 °C was not statistically analyzed, since they did not live the entire duration of the experiment, and therefore could not accurately be compared. However, for the time period that they were alive, it was noted that their growth rate was much higher than that of the control. There was also a noticeable difference in hatching time, when comparing the control to the frog eggs placed at 26 °C.

Our results partly refuted our hypothesis that the frogs placed in the intermediate temperature would have a higher growth rate and an earlier hatching than the frogs placed in the two extremes. In contrast, we found that the frogs placed in the warmest temperature, 26° C hatched first, and also had the highest growth rate. However, these frogs died within two days of hatching, thus supporting Huey (2004) who found that temperatures of approximately 27 °C and higher to be detrimental to the frog survival.

One reason why an increase in temperature is detrimental to the frog’s survival is because as the temperature of water increases, its ability to hold oxygen decreases (Ward 2003). In order to survive, most organisms need dissolved oxygen levels of 5ppm or higher (Washington University 2004). This was a problem in our experiment. The warmer the water was, the faster that its oxygen level dropped. When we first placed the water into the four containers, it had a dissolved oxygen level of 11.2ppm. However, after the first weekend, the eggs placed in the water with a temperature of 26°C had already hatched and had a dissolved oxygen level of 0.97ppm. This rapid decrease in the oxygen content may also have been due to the increased activity level of the frogs after hatching. The water in the container was quickly changed in an attempt to save the frogs. However, the frogs were all dead by the next afternoon.

Another problem that we encountered during our research, which may have affected our data, was a malfunction in the heating unit of the 21 °C tank, which caused the temperature in the tank to drop to 10 °C. It remained at this temperature for approximately three days until the problem was corrected. If this had not occurred, we predict that the frogs would have hatched a few days sooner and would have had an even higher growth rate, thus showing an even stronger

correlation between temperature and developmental rate.

The biggest problem that we encountered during our research was not having enough data to properly analyze our results. This occurred because instead of taking multiple random measurements, we only recorded the average size and hatching time of our frogs. This turned out to be highly problematic because most statistical tests require multiple measurements. For this reason we were not able to statistically analyze the hatching time of our frogs, since no test is available for compare only averages.

There are many opportunities to conduct further research on this subject. First, our experiment could be repeated taking multiple random measurements instead of just averages. This would produce more accurate data and allow for the use of more statistical tests. Also, this experiment could be conducted for a longer period of time, comparing all the major developmental stages of the frog, as well as the life span of the frogs in each of the temperature groups. After death, the frogs could also be dissected to determine if temperature had any effects on organ development. Our experiment could also be performed using other amphibians such as salamanders. This additional research could help determine if temperature plays a significant role in the development of amphibians, and if so, help humans predict what effects global warming may have on amphibians.

## ACKNOWLEDGEMENTS

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## LITERATURE CITED

- Censky, E., C. J. McCoy and A. Hulse. 2001. *Amphibians and Reptiles of Pennsylvania and the Northeast*. Cornell University Press, Ithaca, New York, USA
- Halliday, T and K. Adler. *Declining Amphibian Populations: The Causes and Implications of a Growing Worldwide Threat*. Pages 30-31. *Firefly Encyclopedia of Reptiles and Amphibians*. Firefly Books LTD, Toronto, Canada.
- Huey, R.B. 2003. *Temperature-General Effects*. <<http://www.mikeepstein.com/msm/do.PDF>> . Accessed 2004 April, 21.
- Mayer, R. 2001. *Connections in Environmental Science*. McGraw-Hill, New York, USA
- Ward, M. 2003. *Spring Peeper Project*. <<http://www.shrhs.org/watershed/cp%20%20spring%20peepers%20paper.htm>>. Accessed 2004 April, 21.
- Washington University. *Dissolved Oxygen*. <<http://washington.edu/rbhuey/2003%20Temperature%201.PDF>> . Accessed 2004 April, 21.
- Zar, J.H. 1984. *Biostatistical Analysis*, 2<sup>nd</sup> edition, Prentice-Hall, Engelwood Cliffs, New Jersey, USA.