

THE EFFECTS OF TEMPERATURE ON THE GROWTH OF MARBLED SALAMANDER (*AMBYSTOMA OPACUM*) LARVAE

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ABSTRACT

Our study showed that temperature affects the growth rates of Marbled salamander (*Ambystoma opacum*) larvae. Average larval body size varied significantly among the different temperature treatments. We only observed growth at the middle temperature of 15° C, while the larvae in the two extreme temperatures (5° C and 21° C) continued to decrease in average body size. This continuous decrease in body size may have resulted from unexpected external stressors. However, we concluded that Marbled salamander larvae may be best suited to the mid-temperature range of 15° C.

INTRODUCTION

The Marbled salamander, *Ambystoma opacum*, inhabits vernal ponds and deciduous woodlands throughout much of the eastern coast of the United States (Conant, 1991). Its habitat can range from dry areas such as hillsides to swampy bogs. The adults are one of a few species which lay their eggs in vernal ponds in the late summer and early autumn. In late autumn the eggs hatch, while covered with water, and the larvae become active throughout the winter. The larvae are three-quarters of an inch long and dark colored when hatched. By early spring they grow to be about an inch and a half long (Shaffer, 1991). They have fully expanded gills at the base of their heads (Tyning, 1990). The larvae are relatively easy to find in vernal ponds, where they can be found in late autumn to early spring resting on leaf litter in shallow areas. They are often the only larval amphibians in the pond at this time of the year. During the day they rest at the bottom of the ponds and feed on leaf litter. At night they swim and drift at the various levels of the pond. Marbled salamander larvae also feed on *Daphnia*, earthworms and other small invertebrates.

Normally, the larvae metamorphose in the spring around May or June and then become terrestrial. Since metamorphosis occurs when temperatures are rising, we asked whether temperature affects the rate at which it occurs. Our working hypothesis was that there will be an increase in the growth rate of marbled salamander larvae when there is an increase in the temperature of their environment. In contrast, our null hypothesis is that the growth rate of marbled salamander larvae is independent of temperature. We hope that this study will increase our understanding of how temperature affects the growth rate of *Ambystoma opacum* larvae. This knowledge may increase our ability to wisely manage and protect populations of this species.

FIELD SITE

We collected marbled salamander larvae at a large vernal pond located along Petersburg Pike about 5 miles from Warm Springs Road (near Huntingdon, PA). The temperature of the water was 7° C on our first sampling day, March 17, 1998. Brandon Staub, who is a student currently doing research at this vernal pond, provided us with additional information. He measured the pH in three different areas of the pond on April 15, 1998, and recorded pH values of 4.25, 4.29 and 4.29. This indicated that the chemical composition of the water was acidic. The water temperature on this day was 13.5° C. This shows that the water temperature of the vernal pond had nearly doubled since our first sampling day. Besides this information, we were unable to find any documented information on the chemical composition or physical characteristics of this local vernal pond.

METHODS AND MATERIALS

On March 17th and 18th, 1998, 50 marbled salamander larvae were collected with dip nets and were brought immediately back to the laboratory. The vernal pond water in which the larvae were found was used for all experimental trials.

Five larvae were placed in each of nine 5-gallon aquaria. Three aquaria were kept at each of three temperatures: 5° C, 15° C and 21° C, and regulated on a 12 hour light/dark cycle. To maintain the various temperatures we used two environmental control chambers and the Biology Department refrigerator. The two environmental control chambers were set at 21° C and 15° C. The Biology Department refrigerator was preset at 5° C.

The larvae were fed three types of food at 3-day intervals. Initially we fed the larvae frozen brine shrimp. On a feeding day, the brine shrimp were weighed on an analytical scale and diluted in 50 mL of water. Each tank received 5 mL brine shrimp solution, estimated with a graduated cylinder, during each of the first three feeding days. During the next two feeding days, this diet was supplemented with live water fleas (six adult *Daphnia magna* per aquarium per day). Since this diet did not appear to be sufficient to promote growth in the larvae, the diet was again changed to include larger prey, i.e., freshwater shrimp (*Gammarus minus*) collected from Warm Springs (Huntingdon County, PA). The larvae were fed the same number of amphipods per aquarium during the remaining feeding days.

Body size was estimated by calculating the "square area" of each larvae. To do this, we placed each larva onto a piece of paper, and with a pen marked the length of the body (head to tail) and the width of the head near the gills. The square area was calculated by multiplying body length by head width. We made these measurements for all 45 salamanders in the nine aquaria on four different days. The square areas of all of the larvae in each aquarium were averaged during each measurement day. One-way analysis of variance (Minitab software) was used to test whether significant differences in larval square area existed within temperature treatments (among aquaria) and among temperature treatments during each of four measurement day.

RESULTS

Larval sizes at three different temperatures over four measurements days are summarized in Table 1. Significant differences in larval size among aquaria kept at the same temperature occurred only during the first measurement day (day 1) at 4.5-5.5° C. (Table 2).

Table 1: Average square area (\pm standard deviation) of larval *Ambystoma opacum*

	Lowest Temp (4.5-5.5° C) body size (cm ²)	Middle Temp (15-16° C) body size (cm ²)	Highest Temp (20-21° C) body size (cm ²)
Day 1	2.25 \pm 0.44	2.08 \pm 0.46	2.07 \pm 0.35
Day 2	1.85 \pm 0.25	1.69 \pm 0.38	1.68 \pm 0.27
Day 3	1.84 \pm 0.22	1.74 \pm 0.33	1.58 \pm 0.22
Day 4	1.84 \pm 0.41	1.70 \pm 0.31	1.47 \pm 0.19

Table 2: Comparisons of larval body size (square area) among aquaria at the same temperature and among temperature treatments for each measurement day (one-way ANOVA results, $df=2$).

	Lowest Temp (4.5-5.5° C) P value, F value	Middle Temp (15-16° C) P value, F value	Highest Temp (20-21° C) P value, F value	All Temp for each measurement day P value, F value
Day 1	0.017**, 5.78	0.714, 0.35	0.430, 0.91	0.394, 0.95
Day 2	0.570, 0.59	0.319, 1.26	0.108, 2.7	0.230, 1.52
Day 3	0.620, 0.50	0.517, 0.57	0.639, 0.46	0.034**, 3.67
Day 4	0.476, 0.79	0.674, 0.41	0.878, 0.13	0.002**, 7.0

** Indicates significant values ($P < 0.05$).

Disregarding the heterogenous data for day 1 at 4.5-5.5° C, we combined all of the data collected at each temperature. We combined all fifteen data measurements of the larvae in the lowest temperatures, and we compared it with the fifteen measurements taken in each of the middle and the highest temperature treatments. On Day 3 and 4, we observed a significant variation in the square body size of the larvae at the different temperatures (Table 2). This variation was greater on Day 4, as indicated by a more significant P value (see Table 1.)

DISCUSSION

Our results were unexpected. First, overall the larvae shrank, rather than increased in body size during the experiment. After analyzing our results, we rejected both our null and working hypotheses. We predicted that the larvae at the higher temperatures would have a higher growth rate, because an increase in metabolic rate at higher water temperatures would cause the larvae to eat more food and thus grow at a faster rate. Our results indicated on Day 3 and 4 that there was a significant difference in body size among the larvae maintained at different temperatures [Day 3: $P = 0.034$; Day 4: $P = 0.002$]. By looking at Table 1, we can see that the larvae at the highest temperature maintained a very low average square area throughout the experiment. The lowest temperature group also displayed a pattern of de-growth. However, the larvae in the mid temperature range showed the greatest increase in average body size. This may indicate that the larvae were best suited to this temperature. The highest temperature setting of 21° C may have been too stressful for the metamorphosing larvae. Perhaps the acclimation period of only one day given to the larvae to adjust from 7° C to 21° C was too abrupt for the larvae to suitably respond to their new environment. The cooler temperature (which was actually colder than their natural habitat) may have lowered the metabolic rate and thus the growth rate of the larvae relative to that observed at the "optimal" temperature of 15° C.

Our unexpected results may have been due to additional stressors we placed on the larvae. One of our major concerns was the stressful conditions of our measurement process. We removed the larvae from their aquatic environment and placed them on dry pieces of paper. The larvae appeared to be

stressed when we removed them from the water. We tried to quickly perform the measurement, but the behavior of the larvae made this task difficult. Once the larvae were placed back into the tanks, they displayed unusual, lethargic behavior. After a short time period, the larvae began to display normal movement and activity. In addition, we discovered that our initial food source was not being consumed by the larvae. Due to the lack of nutrients, the larvae were not obtaining enough energy and may have been forced to allocate their energy to survival and maintenance rather than growth. Furthermore, other additional stressors that were imposed on the larvae could have affected our results.

If further research were to be conducted on this topic, a greater understanding of variables affecting the growth of marbled salamander larvae would be necessary. We feel that there were too many external factors affecting the growth of the larvae. A more in-depth, long term study with better procedures would be necessary to eliminate such factors.

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