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The effect of oxygen levels on the metabolism and growth in *Ambystoma macrodactylum*

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MHS Honors Biology

Question: How does dissolved oxygen affect the metabolism and growth of *Ambystoma macrodactylum*?

Abstract: The goal of this experiment was to show how dissolved oxygen in water affects growth in salamanders. Previous research showed that environmental stimuli can alter the bodily processes of amphibians. Based on this information, it was hypothesized that high dissolved oxygen levels in water would increase salamander growth. Two groups of three salamanders each were raised in different containers of equal size. The water in one group was bubbled with oxygen for ten minutes each day, while the water in the other group was not altered. The growth of the salamanders in each group was recorded. The non-oxygenated group grew to 54 millimeters long, and the oxygenated group grew to be 52 millimeters long. The data demonstrated that lower oxygen levels support salamander growth, but because of the very small difference in length, it is possible the oxygen levels had no effect on growth in the salamanders. The original hypothesis was not supported.

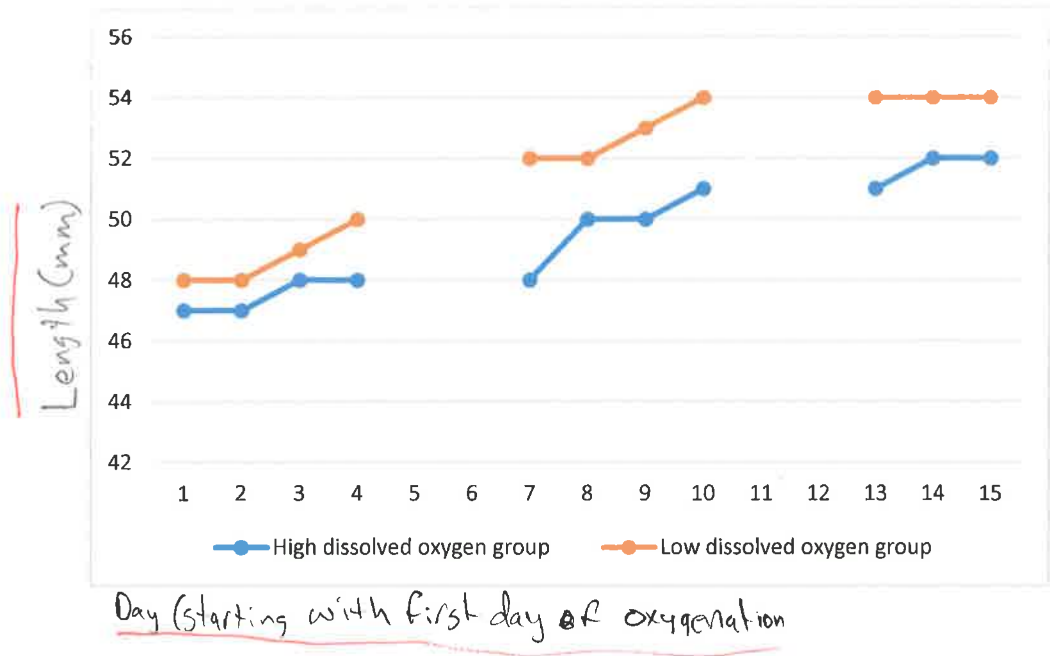
Introduction: The purpose of this experiment was to determine the effect oxygen levels in long-toed salamanders' aquatic environments had on their metabolic rates, and thus eating habits and growth. Metabolism measures and animals ability to turn food into energy, which can be used to grow. Studies have shown that external factors can have a large impact on the internal bodily process of amphibians. However, less research has been conducted to demonstrate the effects these factors have growth in salamanders. Various studies have shown that various environmental factors can influence amphibians' metabolic rates. One study found that temperature can alter the metabolic rates of one species of salamander, *Eurycea bislineata* (Fitzpatrick, 2003). Other experiments have shown that amphibians, as well as reptiles and fish, can change bodily processes, including suppressing their metabolic rates, in extreme oxygen levels (Bickler and Buck, 2007). Based on this information I hypothesized that if high oxygen levels increase salamander's metabolic rates, and if I raise one group of salamanders in high oxygen containing water and another in low-oxygen levels, then the salamanders in the high oxygen containing water with oxygen will eat and grow more than the salamanders living in lower oxygen levels.

Materials/Methods: This experiment required two separate groups of salamanders and two containers of equal size to keep them in. The containers were filled with small rocks along the bottom and one larger rock for the salamanders to crawl onto after metamorphosis. De-chlorinated water was filled to just below the height of the large rock. Daphnia, garden worms, and blood worms were required to feed the salamanders. Oxygen bubblers and a machine to measure dissolved oxygen were required to take measurements.

The salamanders were collected as eggs and stored until they hatched. Once they hatched, they were fed daphnia until they were approximately 45 days old. At that point they were fed blood worms. At day 75 the oxygen levels in two groups of salamanders (three in each group) were first altered. One group had oxygen bubbled through their container for ten minutes a day. The oxygen levels in the other group of salamanders were not altered. Then both groups of salamanders were fed an equal amount of food, which consisted of blood worms, garden worms, and tadpoles. Their growth was monitored and recorded every day. The experiment ended when the salamanders were 90 days old.

Data: (See Appendix Table 1 for data table)

Figure 1. Growth of salamanders in high and low levels of dissolved oxygen.



Discussion/Analysis: The salamanders in the low oxygen levels grew more than the salamanders in the high oxygen levels. By the end of the experiment the largest non-oxygenated salamander was 54 millimeters (mm), but the largest salamander in the group with oxygenated water was only 52mm. The results indicate that higher concentrations of dissolved oxygen inhibited growth of salamanders in this experiment. It is also possible that lower levels of dissolved oxygen promoted the growth of the salamanders in this experiment. However, a difference of only 2 mm in growth is very small. This difference may have been caused by other uncontrolled environmental or genetic factors. It is possible that dissolved oxygen levels did not affect the growth of the salamanders at all, but other factors influenced their growth. The salamanders in the non-oxygenated group were slightly bigger at the start of the experiment than those in the oxygenated group. Their size at the end of the experiment may just have been a function of natural, but different, growth patterns in the salamanders in the different groups.

It was also noted during this experiment that the salamanders in the non-oxygenated group metamorphosed earlier than those in the oxygenated group. This indicates that higher oxygen levels prolong the start of metamorphosis. However, this may have also been a function of genetics, or other uncontrolled variables in the experiment.

One possible variable that may have contributed to the difference in size of the salamanders is the loss of two of the three salamanders from the group with oxygenated water. One escaped from the container and the other is suspected to have died through improper formation of the lungs while it was metamorphosing. The difference in the number of salamanders in the two containers for nine days during the experiment likely impacted the remaining salamander's growth, as well as the dissolved oxygen levels. However, this would have increased the dissolved oxygen concentration in the water, which, since it the deaths occurred in the high-oxygen group, should not have affected the results. The deaths may also be indicative of other unknown substances in the water that affected the growth of the salamanders.

It is also possible that bubbling the water had relatively little effect on the salamanders. When taking measurements of the dissolved oxygen levels in each container, it was noted that oxygen levels were highest after the water had been changed. The number of times the water was changed per week in each container appeared to affect the levels of dissolved oxygen more than bubbling oxygen through the water did. The number of times the water was changed per week for each group of salamanders was not controlled in this experiment. One group may have had their water changed more frequently than the other, making them have higher concentrations of dissolved oxygen. Because dissolved oxygen measurements were not taken after the first week of oxygenation, the dissolved oxygen levels in the non-oxygenated salamanders may have actually been higher than the oxygenated group. If this were the case then the initial hypothesis would have been supported.

Other variables may have also contributed to the results of this experiment. The volume of water for the container for both of the groups was not regulated. Although not a significant factor, the difference in the volume of water in each container still may have had some effect on the growth of the salamanders. There was also a very small sample size for this experiment. With such a small sample size, natural factors, such as the deaths of some salamanders, have a larger impact on the experiment than they would with a larger sample size. Furthermore, during the experiment there was a shortage of food for the salamanders. They were not fed as much as they needed for several days. This period could have occurred during a critical time in salamander growth, and affected the results of the experiment. Oxygen levels were also first altered late in the salamanders' lives. The experiment may have begun too late, and not lasted long enough, for the possible effects of dissolved oxygen on salamanders to be observed. Observation and oxygenation did not occur every day during the experiment, as well, which may have made the results less accurate.

There were too many uncontrolled variables in this experiment for it to be an accurate representation of how dissolved oxygen affects growth and metabolism in salamanders. If this experiment were to be repeated these variables would be more controlled. Dissolved oxygen levels would start to be altered as soon as the salamander eggs hatched. A larger sample size would be beneficial in minimizing the effect outlying groups and of natural and uncontrollable variables, such as death of salamanders, in the experiment. Each salamander would be fed an adequate and specific amount of food every day. The water in the high-oxygen level group would be oxygenated each day, and the size of each salamander would be recorded. It would also have been beneficial to have separated the groups and begun oxygenation sooner, during periods of more significant growth.

While not being completely accurate, this experiment raise further questions about salamander growth. Growth in salamander may be affected by other environmental factors, besides oxygen levels. Another hypothesis to test could be: if salamanders grow faster in cooler environments than in warmer environments, and if I raise one group of salamanders between 40 and 50 degrees Fahrenheit, and another group of salamanders between 60 and 70 degrees Fahrenheit, then the salamanders in the cooler climate will grow to be longer than those in the warmer climate.

Works Cited

Fitzpatrick, L. (2003). Download PDFs. Retrieved April 10, 2015, from http://www.sciencedirect.com/science/article/pii/0300962973900832

Bickler, P., & Buck, L. (2007). Hypoxia Tolerance in Reptiles, Amphibians, and Fishes: Life with Variable Oxygen Availability. Retrieved April 10, 2015, from http://www.annualreviews.org/doi/abs/10.1146/annurev.physiol.69.031905.162529

Appendix

Table 1

Day	Length of Salamanders in High Dissolved Oxygen Levels	Length of Salamanders in Low Dissolved Oxygen Levels
1	47	48
2	47	48
3	48	49
4	48	50
5		
6		
7	48	52
8	50	52
9	50	53
10	51	54
11		
12		
13	51	54
14	52	54
15	52	54