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# The Effects of Salinity Increase on Spring Peeper Tadpole (*Pseudacris crucifer*) Growth and Development in the Douglas Lake Area

## Abstract

The physiology of frogs makes them good indicators of their surrounding environment. Their permeable skin allows for absorption of needed solutes, but also makes them vulnerable to absorbing excess chloride as well. Industrial and human wastewater and salt run-off from roads during the winter in northern Lower Michigan is a concern for many freshwater environments. As chloride concentrations increase, frogs are unable to osmoregulate. The effect of increased salinity on the growth and development of spring peeper (*Pseudacris crucifer*) tadpoles in the Douglas Lake area was tested over the course of two weeks. It was hypothesized that increased salinity would osmotically stress the tadpoles and predicted that the increased salinity would decrease tadpole growth. Average tadpole growth ranged from 0.46 cm to 1.015 cm (p=0.851). It was concluded that salinity of 6 to 8 parts per million (parts per million of chloride will be referred to as ppm from here on) do not have an effect on spring peeper tadpole growth in northern Lower Michigan. An observational study of four Douglas Lake area ponds and marshes was also done. It was hypothesized that increased chloride levels would affect tadpole species richness and predicted that increased chloride levels will decrease the number of species present in the pond or marsh. Sedge Point pond had 1 ppm with 2 tadpole species; Grapevine Trail pond had 6.0 ppm with no tadpole species; Burt Lake area pond had 4.9 ppm with 1 tadpole species; and Cheboygan area marsh had 225.5 ppm with 3 tadpole species. No statistical tests were performed, but by observation it was concluded that salinity does not have an effect on Douglas Lake area tadpole species richness. Although we saw no difference in tadpole growth at 6, 7, and 8 ppm, further studies must be done to determine what chloride levels will begin to affect tadpole development.

## Introduction

Frogs, common in nearly all aquatic environments in Michigan, are extremely sensitive to changes in their habitats. They are a valuable indicator of the health of freshwater ecosystems because their populations tend to quickly decrease if their environment is disrupted (Hutchins et al. 2003). Part of their sensitivity to environmental changes, such as increased salinity concentrations, occurs because they cannot osmoregulate (Gomez-Mestre et al. 2004). In all stages of life frogs have a permeable layer of skin which allows water and solutes to be absorbed and excreted (Hutchins et al. 2003). Water permeates their skin to keep them hydrated, but with increasing levels of pollutants in their waters, frogs are constantly absorbing potentially lethal levels of chlorides and other chemicals as well (Hutchins et al. 2003). Frogs represent a major portion of the species within terrestrial ecosystems and are linked with the small invertebrates they eat and the larger vertebrates that eat them (Halliday 2002). The declining population of frogs has become a major concern for scientists around the world.

Tadpoles also play a major role in ecosystems. They ingest a large portion of algae while metamorphosing and are the staple food for many insects, snakes and small mammals (Halliday 2002). Similar to frogs, tadpoles exhibit notable sensitivity when exposed to a dehydrating environment (Hutchins et al. 2003). Studies show that tadpoles under a salinity of 140 ppm showed a slower developmental rate, metamorphosing between 4 to 9 days later than the freshwater control (Chinathamby et al. 2006). Those under a salinity of greater than 192.5 ppm showed almost 100 percent mortality (Dickman 2002). This decrease in development and increase in mortality may be linked to a decrease in the thyroid growth hormone in tadpoles (Gomez-Mestre et al. 2004). This difference in growth could also be related to a physiological trade-off between the regulation of solutes and development (Hutchins et al. 2003). Disruption of tadpole development has the potential to affect many levels of the food web.

The many amphibian species around the Great Lakes region are at risk as their aquatic and terrestrial environments change. During the past 100 years there has been a significant increase in the amount of chlorides dumped into freshwater systems from industrial and human wastewater (Kormondy 1996). On average, northern Lower Michigan receives 203.5 inches of snow (World Almanac 2007). To melt this snow large amounts of salt are applied to the roads each winter. The salt run-off each spring adds to the chloride concentrations in freshwater environments. The increased salinity caused by human development, industries, and run-off are having a significant and detrimental effect on frog and tadpole populations in the Great Lakes region (Dickman 2002).

The objective of our experiment was to evaluate the effects of salinity on tadpole growth and development, specifically in the spring peeper tadpole (*Pseudacris crucifer)*. This species was chosen because of its abundance throughout the United States and its sensitivity to environmental changes. We hypothesized that increased salinity would osmotically stress the tadpoles. As a result of osmotic stress, we predicted that tadpole growth would decrease as salinity was increased.

An observational study of four ponds around the Douglas Lake area was also conducted to evaluate salinity and presence or absence of tadpole species. We hypothesized that salinity will influence the number of tadpole species present. In ponds of high salinity, we predicted that the variety of tadpole species would be less than areas with lower concentrations.

## Methods and Materials

### *Study Species*

Spring peeper frogs (*Pseudacris crucifer*) live in wet, wooded areas protected from wind and often lay their eggs in ephemeral ponds. Female spring peepers lay eggs from March until May and the tadpoles develop quickly into adults by July (Nova Scotia Frogs). These factors made the spring peeper tadpole an ideal species for this research given the geographic area and time constraints.

### *Mesocosm Experiment*

Spring peeper tadpoles were collected from a small ephemeral pond off the shore of Douglas Lake, near Pellston, MI. As a control, 3.5 liters of Douglas Lake water at 6 ppm were added to five plastic containers. For the experimental groups, Douglas Lake water was adjusted to 7 ppm and 8 ppm by adding sodium chloride (NaCl). When deciding on the two experimental group concentrations, consideration was given to the time allotted for completion of the experiment and to the well-being of the tadpoles. Transferring tadpoles into waters of high salinity may shock, stress and even kill the tadpoles because of their inability to acclimate to the given conditions. Therefore, it was decided to increase the chloride concentration of water from Douglas Lake by only 1 and 2 ppm. Randomly captured tadpoles were measured in centimeters and five were randomly placed into each of 15 separate plastic containers. To provide food for the tadpoles, vegetation from the pond was placed into each container. In order to keep other conditions constant, the 15 containers were left submerged halfway in the pond where the tadpoles were found for the duration of the experiment. A thin mesh net was placed over all the containers to prevent predator disturbances.

During our experimental time period, we checked on our mesocosms every 3 to 4 days to ensure that water levels stayed constant. Since chloride does not evaporate 300 mL of Douglas Lake water was added to all containers when water levels became low to keep concentrations constant.

After an incubation period of 14 days, the lengths of the tadpoles were measured again. Tadpole growth was calculated by finding the differences between the original tadpole length averages and final tadpole length averages. Data were checked for normality with Q-Q plots and a one-way ANOVA test was used to determine whether the average growth of tadpoles in the three groups were statistically different at an alpha of 0.05 (SPSS version 14.0).

*Observational Study*

Three ponds and one marsh that were known to have different salinity levels and were also home to various frogs and tadpoles were selected around the Douglas Lake area for observation. Water samples were taken from each site to determine precise chloride levels. While at each pond, the number of tadpole species found in the pond was recorded.

**Results**

*Mesocosms Experiment*

Average tadpole growth was not significantly different between treatment groups (p=0.851, Figure 1). In the control (n=5) average tadpole growth was 0.656 cm (x-bar ± 0.215); at 7ppm (n=5) average growth was 0.56 cm (x-bar ± 0.213); and at 8 ppm (n=5) average growth was 0.693 cm (x-bar ± 0.137).

*Observational Study*

Sedge Point pond on Douglas Lake was calculated to have 1.13 ppm and 3 tadpole species; Grapevine Point pond had 6.0 ppm and no tadpole species; Burt Lake Road area pond had 4.9 ppm and 1 tadpole species; and Cheboygan area marsh had chloride levels of 225.5 ppm and 3 tadpole species. No statistical tests were done.

**Discussion**

Given that literature suggests a negative relationship between tadpole growth and salinity level, there was strong evidence to support the idea that higher salinity would stunt tadpole growth. However, we failed to reject our first null hypothesis and concluded that salinities of 6 to 8 ppm have no effect on spring peeper tadpole growth in northern Lower Michigan. There are many possible reasons why no difference was apparent.

In a previous study, salinity of 140 ppm or greater have been recorded to have serious effects on tadpole growth and development (Chinathamby et al. 2006); however, we tested at much lower levels than this. This was because tadpoles must be acclimated to their environment prior to testing to ensure that shock is not the cause of stunted growth or mortality. Due to time constraints, we were unable to acclimate our tadpoles, thus we were unable to subject them to high levels of chloride. On the other hand, it may have been worthwhile to test at low salinity with only 1 and 2 ppm increments of increases because most previous studies have only focused on extreme salinity increases. Few studies have focused on the effects of small salinity changes on tadpoles. Studying smaller increments of salinity change can give a more detailed dose response curve of tadpole growth at each concentration. For instance, salinity increase and tadpole growth may not be a linear relationship. Tadpoles may be able to survive and grow in a range of salinities until a certain point at which they can no longer persist. Extensive studies should be done to test slight increases in salt concentrations throughout a broad range on tadpole growth. Knowing exactly how salinity affects tadpoles at every concentration can bring us closer to generating a plan for protecting tadpoles and frogs.

Mortality in tadpoles was also noted and considered when performing statistical analysis of tadpole growth. There were a total of 3 tadpole deaths in the control group, 13 deaths in the 7 ppm group and 1 death in the 8 ppm group, out of a total of 25 tadpoles per group. When calculating tadpole growth averages, deaths were not counted into the average length grown per tadpole in each of the three groups.

Since two entire containers of tadpoles were lost, the deaths were most likely caused by a predator getting into the container. Tadpoles may have also escaped due to the large amount of rainfall experienced during the time of the experiment, which may have flooded the containers.

Size biased mortality, in which the smallest tadpoles in each treatment group died, could have also played a role in the deaths. Smaller sized tadpoles may have become dehydrated too quickly causing survivorship to occur in only those tadpoles (most likely large tadpoles) that could handle the salinity. If size selectivity was a factor of the deaths, average length calculated may not have been the same average if all tadpoles had survived. This type of size biased mortality may have shifted the average growth length to a higher amount than it realistically was. Although unlikely because of the small increase in salinity, mortality in the smallest tadpoles could also help to explain why no difference was seen in average growth between the groups as salinity was increased.

Based on the data collected from our observational comparative study, we failed to reject our second null hypothesis because we did not see a relationship between salinity and species richness of tadpoles. This data suggests that tadpoles are capable of surviving in higher salinity than at the levels for which we tested.

If this experiment were to be repeated, we would suggest varying the salinity of experimental groups and increasing the range of concentrations. Based on previous study results, we were cautious in raising the salinity much higher than the natural environment of the tadpole because of the stress and dehydration. However, other experimental studies have found that tadpoles can survive and grow under substantial concentrations of chloride (Sanzo 2005). Realistic concentrations of chloride in natural ponds and wetlands can reach up to 18,000 ppm and still contain tadpoles (Sanzo 2005). Although no amphibians are found in pure salt water (35,000 ppm of chloride) many have adapted to live in extremely brackish environments (Halliday 2002). This is not to say that high salinity has no effect on tadpole growth and development. Research shows an undeniable decrease in frog populations all over the world due to the addition of chlorides and other pollutants (Halliday 2002). Although our data may not have supported this fact previous studies have shown that salinity does have a negative relationship on tadpole growth and development. We would suggest that this test be repeated again with increased salinities to confirm the effects of salinity on spring peeper tadpole growth and development.

Spring peeper tadpoles can sustain a much higher salinity than we expected. Although we found no relationship between increased salinity and tadpole growth, nor did we find a relationship between salinity and tadpole species richness, we cannot deny previous studies that say such relationships do exist. Frog populations are decreasing all over the world due to human impact. The disappearance of tadpoles and frogs could have detrimental effects on the biodiversity and trophic levels in nearly all terrestrial ecosystems (Halliday 2002). These facts alone are enough to encourage further research on the effects of increased salinity due to humans on tadpole growth and development.

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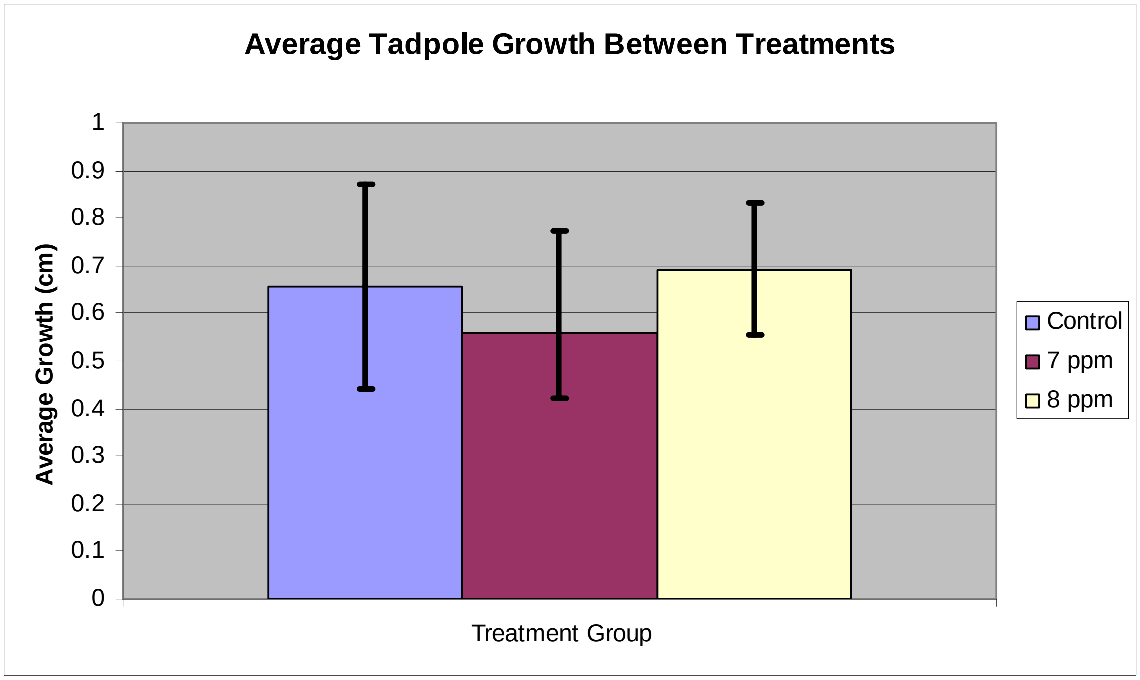


Figure 1. This graph shows the relationship between treatment groups (Control, 7 ppm and 8 ppm) and average growth (in centimeters) of the tadpoles in those treatment groups. Control tadpoles grew an average of 0.656 cm, 7 ppm tadpoles grew an average of 0.560 and 8 ppm tadpoles grew an average of 0.693. The error bars represent plus/minus 1 standard deviation of each mean growth (Control SD=0.216, 7ppm SD=0.214 and 8 ppm SD=0.137)