Road Salt: At Fault? The Effect of Chloride Concentration on the Mass of *Ambystoma maculatum* Egg Masses

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Abstract

Ambystoma maculatum, commonly named spotted salamanders, serve as bioindicators because of their sensitivity to ecological changes; thus, a variety of environmental factors affect their eggs, including chloride concentration. Past studies found that an increase in chloride concentration results in a significant decrease in the mass of *Ambystoma maculatum* egg clutches. We hypothesized that chloride concentration would negatively correlate to the mass of spotted salamander egg clutches. We conducted our study in Lorton, Virginia, at Elizabeth Hartwell Mason Neck National Wildlife Refuge, during the springs of 2018 and 2019. The refuge contains four vernal pools, each of which we divided into grids with 1 by 1-meter cells. We searched each cell for egg masses and measured the chloride concentrations of 25 random cells in each pool. We then stratified each pool into 5 by 5-meter squares and calculated the average chloride concentration and the average mass of all observed egg masses within each stratum. Using a *t*-test for the slope of the least-squares regression line, we found that the 2019 data was not statistically significant at the 0.05 significance level, but the 2018 data was significant. One should note that there were multiple environmental differences between the two years, including the lengthened hydroperiod in 2019. Due to these conflicting results, we could not determine if chloride concentration correlates with average *Ambystoma maculatum* egg clutch mass.

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Crucial to the health of the biosphere, amphibians make up a large part of many ecosystems and serve as excellent bioindicators for the ecosystems they inhabit because they live in both aquatic and terrestrial habitats, and react strongly to pollution (Blaustein & Wake, 1990). Unfortunately, around the globe, researchers have found a decline in the populations of a variety of amphibian species in recent history. In Italy, D'Amen and Bombi (2009) found that of the 19 species of amphibians inhabiting Italy the researchers studied, 52.63% of these species' populations declined at a rate of 15-20% between 1985 and 2004. In the Mexican states of Oaxaca, Guerrero, and Chiapas, researchers conducting a study between June 19 and July 25, 2000, found that 24 previously recorded species of amphibians, including 11 endemic species, had disappeared from those regions (Lips, Mendelson, Muñoz-Alonso, Canseco-Márquez, & Mulcahy, 2004). Amphibian decline also appears in the United States. In Colorado, populations of monitored boreal toads and their egg masses declined from previous years in both 1996 and 1999 (Muths, Corn, Pessier, & Green, 2003). Annual survival rates for toads also severely declined, averaging 78% from 1991 to 1994 but averaging 45% in 1995 (Muths et al., 2003). Between 1998 and 1999, survival rates were at just 3% (Muths et al., 2003). In New Hampshire, adult populations of the salamander *Gyrinophilus porphyriticus* decreased by approximately 50% between 1990 and 2010 (Lowe, 2012). The problem of amphibian decline has also spread near Virginia. Corser (2001) found that the relative abundance of seven green salamander populations in North Carolina declined by approximately 97% between 1970 and 1999. Finally, in Virginia itself, the Virginia Wildlife Action Plan has labeled around 38% of all amphibian species as a Species of Greatest Conservation Need (Sevin & Kleopfer, 2015). In Virginia, 52% of salamander species and 29% of frog species are of conservation concern, and four amphibian species are State Threatened or State Endangered Species (Sevin & Kleopfer, 2015). The U.S. Fish and Wildlife Services has also listed the Shenandoah Salamander as Federally Endangered (Sevin & Kleopfer, 2015). Amphibian population trends are alarming, and researchers have not yet found solutions to the various potential causes of amphibian decline.

A variety of causes contribute to the decline of amphibians. Researchers in Mexico and Colorado found that habitat disturbances and diseases such as chytrid fungi have hurt amphibian populations (Lips et al., 2004; Muths et al., 2003). In Italy, D'Amen and Bombi (2009) identified many factors, particularly climate change, but also solar irradiation and habitat change. Sevin and Kleopfer (2015) also described several factors which negatively affect amphibian populations, such as invasive species, roads, and pollution. In particular, higher chloride concentrations due to increased vernal pool pollution, especially from road deicing salt application, amphibian populations experience heavier negative impacts (Collins & Russell, 2009). Amphibian species richness tends to decrease as chloride concentration increases (Collins & Russell, 2009). Additionally, amphibian species abundance tends to decrease as the concentration of salt-saturated water increases (Hossack et al., 2018). Examining past studies concerning how chloride concentration impacts amphibian development offers researchers insight into trends as they conduct new studies.

Road salt contamination and its effects, including higher chloride concentration and conductivity, can damage the health of vernal pools and the organisms living in them. Vernal pools consist mostly of snowmelt and storm runoff (Turtle, 2000). However, roadside pools may receive runoff containing vast amounts of road salt—researchers have measured chloride concentrations of over 18,000 mg/L in road runoff water in Canada (Collins & Russell, 2009).

This large salt input from the water influx contributes to roadside pools having high chloride concentrations—researchers have reported chloride concentrations of 4000 mg/L in roadside pools in Canada (Collins & Russell, 2009). Vernal pools tend to have small watersheds, so the entering water has little contact with soil buffering systems, the ability of soil to resist pH changes through absorbing and releasing hydrogen ions (Turtle, 2000). Additionally, pools usually have little water to help dilute the pollutants entering the pool (Turtle, 2000); this water quickly evaporates in the summer, intensifying the chloride concentration (Collins & Russell, 2009). These characteristics of vernal pools result in pollutants affecting the aquatic systems in vernal pools more strongly than those in other bodies of water.

Chloride concentration from road salt negatively impacts amphibian embryo survival. Researchers discovered that spotted salamander and green frog embryo survival decreased with increasing chloride concentration (Karraker, Gibbs, & Vonesh, 2008). In a lab study, researchers discovered that spotted salamander embryo survival declined from 84% at a low chloride concentration of 1 mg/L, to 68% at a moderate concentration of 145 mg/L, to just 3% at a high concentration of 945 mg/L (Karraker et al., 2008). In another study, embryo survival also declined as chloride concentration increased—survival was 85%, 80%, 61%, 37%, and 7% in 1, 33, 145, 465, and 945 mg/L chloride concentrations, respectively (Karraker & Ruthig, 2009). Researchers determined the moderate and high concentrations to be the mean and maximum concentrations, respectively, in roadside vernal pools in the Adirondack region of New York, USA, from which they obtained data in 2002 and 2003 (Karraker et al., 2008). Green frog embryo survival declined from 91% to 77% to 41% between concentrations of 1 mg/L, 145 mg/L, and 945 mg/L, respectively (Karraker et al., 2008). Other researchers found that wood frogs and spotted salamanders did not occupy pools with high chloride concentrations of above

945 mg/L (Collins & Russell, 2009). The negative correlation between chloride concentration from road salt and amphibian embryo survival means that amphibian populations decline in pools with a higher influx of salt.

Road salt contamination has been found to affect *Ambystoma maculatum* more than other amphibian species. Researchers found that among five species of amphibians studied (spotted salamanders, wood frogs, spring peepers, green frogs, and American toads), spotted salamanders had the highest chloride-sensitivity, with a median lethal chloride concentration of 1178.2 mg/L (Collins & Russell, 2009). This concentration was the lowest amongst the five species (Collins & Russell, 2009). Other scientists found that road salt negatively affected spotted salamanders more than green frogs (Karraker et al., 2008). As mentioned before, at a chloride concentration of 945 mg/L, only 3% of spotted salamander embryos survived compared to 41% of green frog embryos (Karraker et al., 2008). The researchers also observed that spotted salamander embryos experienced a 16% survival reduction at a chloride concentration of 145 mg/L, indicating that even moderate concentrations of road salt can affect spotted salamander embryos (Karraker et al., 2008). Spotted salamanders breed early in the spring, so high chloride inputs from saline runoff at that time easily affect their embryos and larvae (Collins & Russell, 2009). Additionally, they have a long embryonic stage of five to six weeks, and chemical contamination especially affects amphibian embryos (Karraker et al., 2008; Karraker & Ruthig, 2009). The effects of chloride concentration from road salt on amphibians highlight the need to implement successful methods to curb road salt influx into vernal pools. In addition, researchers need to study ways to repair the damages this influx inflicts on vernal pools and their inhabitants, lest some of these inhabitants become extinct. For species such as *Ambystoma maculatum* that are particularly sensitive to road deicing salt, scientists must research methods to minimize the flow of road salt into their habitats.

Past studies clearly document the negative correlation between chloride concentration and spotted salamander egg mass health. One should note the particular sensitivity of spotted salamanders to higher chloride concentration in comparison to other amphibian species (Karraker et al., 2008; Collins & Russell, 2009; Karraker & Ruthig, 2009). Many researchers have highlighted the environmental significance of the negative correlation between chloride concentration and spotted salamander egg mass health, particularly in indicating vernal pool pollution and amphibian population decline (Karraker et al., 2008; Collins & Russell, 2009). Previous studies have shown that a chloride concentration of 145 mg/L resulted in an 18% decrease in mass and that a chloride concentration of 945 mg/L resulted in a 33% decrease in mass (Karraker & Gibbs, 2011). We predicted that groups of cells with higher chloride concentrations would tend to have a lower average mass of *Ambystoma maculatum* egg masses.

Site Description

We conducted research at the Elizabeth Hartwell Mason Neck National Wildlife Refuge (EHMNNWR) in Lorton, Virginia, located east of the Potomac River and northeast of Occoquan Bay (Google Maps, n.d.; U.S. Fish & Wildlife Services, 2013). The United States Fish and Wildlife Service manages the refuge, which resides approximately 29.0 kilometers to the south of Washington D.C. and has an area of approximately 901.2 hectares (U.S. Fish & Wildlife Services, 2011). It has four vernal pools: Anchorage Constructed (located at 38.623532661°, -77.1877650027°), Anchorage Reference (located at 38.630854916°, -77.1940387833°), High Point II (located at 38.652386700°, -77.1625691500°), and Woodmarsh (located at 38.647459885°, -77.1660876917°). Anchorage Constructed and Anchorage Reference lie adjacent to Anchorage Road, while High Point II lies adjacent to High Point Road. The entire refuge is heavily forested; however, workers previously removed some trees to construct Anchorage Road and communication lines. The refuge contains upland hardwood forest and has 36 known species of trees (U.S. Fish & Wildlife Services, 2013). The vegetation includes Virginia pine (*Pinus virginiana*), oak (*Quercus*), hickory (*Carya*), beech (*Fagus*), and maple (*Aer*) trees (U.S. Fish & Wildlife Services, 2013). Notable species in the refuge include the American bald eagle (*Haliaeetus leucocephalus*), great blue heron (*Ardea herodias*), white-tailed deer (*Odocoileus virginianus*), and spotted salamander (*Ambystoma maculatum*; U.S. Fish & Wildlife Services, 2013).

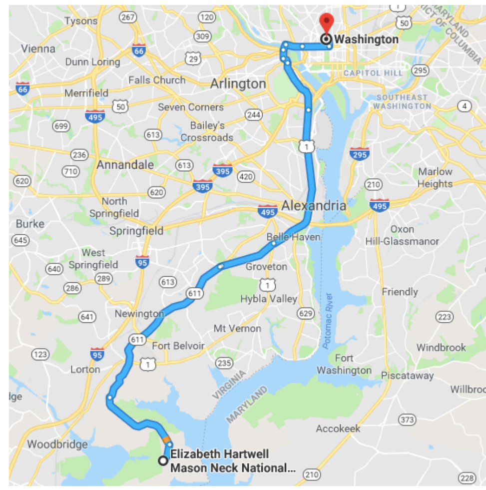

Figure 1. Elizabeth Hartwell Mason Neck National Wildlife Refuge is located approximately 29.0 kilometers southwest of Washington, D.C. (Google Maps, n.d.).

Methods

We began our study on September 26, 2018, when we visited the vernal pools Woodmarsh and High Point II at EHMNNWR. There, we took detailed notes on the vernal pools and the land surrounding them. On the second day of the study, November 26, 2018, we measured the elevation of each of the vernal pools. Prior to measuring the elevation, we divided the pool into a grid of 1 by 1-meter cells. We labeled the rows and columns with flags on the edges of the grid using an alphanumeric system: we labeled rows with letters and label columns with numbers. We then recorded the elevation in each cell using white poles marked with measurements (in centimeters) and an optical survey transit. Lastly, we placed leaf litter bags in certain randomly selected cells for recording macroinvertebrates later on.

We returned to the two sites on March 20, 2019, and March 21, 2019. Using the aforementioned grid system, we searched for egg masses in each cell of the pools and recorded various characteristics of them, including their mass, volume, whether they were cloudy, whether they were attached or free-floating, and whether they had algae. We also looked for and identified macroinvertebrates in the macroinvertebrate bags we had placed in the pools on the previous trip.

Additionally, we collected samples of water from 25 randomly selected cells in test tubes and brought them back to our school, Thomas Jefferson High School for Science and Technology. Using a Vernier Chloride Ion-Selective Electrode (ISE) and a Vernier LabQuest device, we submerged the ISE into each sample of water and held the probe still until the chloride concentration in parts per million stabilized (as indicated on the LabQuest screen). Once the reading stabilized, we recorded the chloride concentration in the corresponding cell of a spreadsheet.

Results

Due to the fact that we were testing for a negative correlation between bivariate data, we used a one-sided *t*-test for the slope of the least-squares regression line. In order to consider the conditions for the statistical test, we used a scatter plot of the data, the respective residual plots, and a histogram of the residuals.

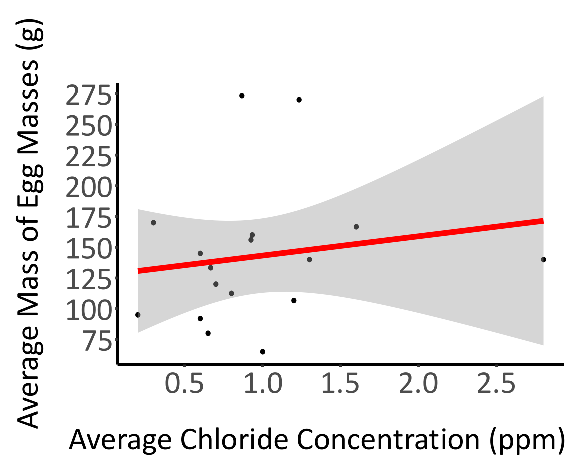

Figure 2. A scatterplot of the 2019 data shows a positive correlation of approximately 0.179. The scatterplot shows an unexpected random scatter. The confidence bands, indicated by the grey area, are wide, showing that the regression may be inaccurate.

To organize the data, we used Microsoft Excel and Google Sheets. Because our data set was limited, we stratified the 1 by 1-meter cells into 5 by 5-meter strata. This was because most cells had one of either chloride or egg mass measurements, but only a few had both. For each stratum, we averaged the chloride concentrations and the mass of the egg clutches, so our observational units were each stratum. We used the statistical software RStudio to create scatter plots and residual plots, analyze the data, and conduct the t-test. To verify the t-test, we used a Texas Instruments TI-84 Plus calculator (version 2.55MP). In our t-test, we used an alpha level of 0.05 and the null hypothesis that average chloride concentration does not correlate with average egg clutch mass. Our alternative hypothesis was that the true slope of the least-squares regression line between chloride concentration and egg clutch mass is less than 0.

The data showed a very weak and positive correlation between average chloride concentration and average egg clutch mass (Figure 2). We found three outliers, with coordinates (2.8, 140), (0.2, 95), and (0.3, 170) using a Tukey bivariate outlier test (Figure 2). Figure 3 shows that the data is not roughly normal. In addition, because the number of residuals below the "residual = 0" line differs from those above the line (Figure 3), the data did not show equal variance and thus follows a random pattern.

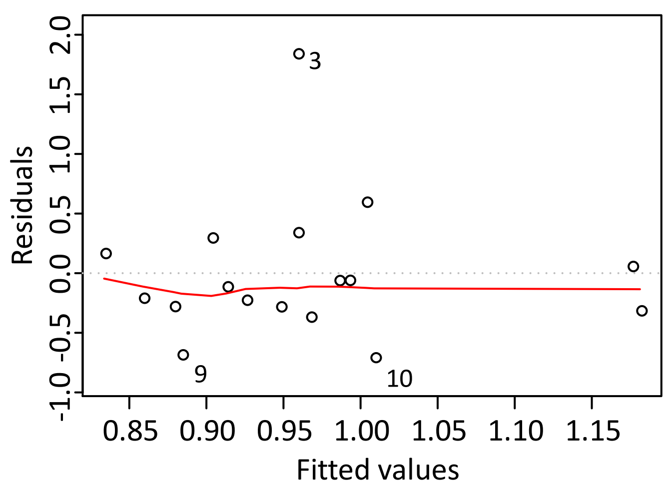

Figure 3. A scatterplot of the residuals for the 2019 data, which appear to be random, as desired.

We found that strata with a higher average chloride concentration did not have a significantly lower average egg clutch mass than other strata. We failed to reject the null hypothesis, $r = 0.179$, $b = 16.353$ grams per ppm, $n = 17$, $t(15) = 0.706$, $p = 0.754 > \alpha = 0.05$. We had no evidence of a negative linear relationship between chloride concentration and the mass of spotted salamander egg clutches for spring of 2019.

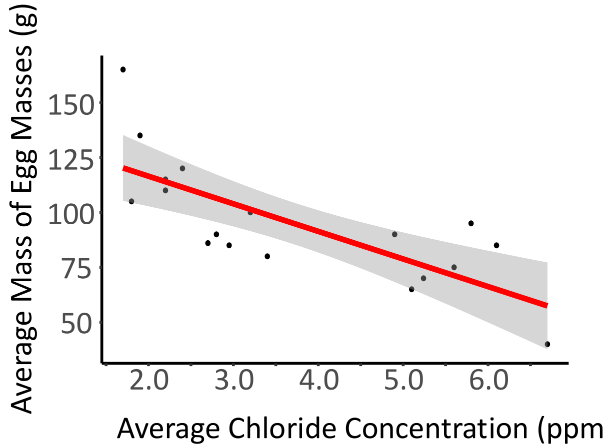

Figure 4. A scatterplot of the 2018 data shows a negative correlation of approximately -0.754. The confidence bands, indicated by the grey area, show that the regression line is likely to be accurate.

Our data seemed to be random (Figure 2), so we decided to analyze data from the spring of 2018 to further investigate. We stratified the data and conducted the t-test in the same manner as the previous procedure.

The data from 2018 showed a negative, moderately strong linear correlation between average chloride concentration and average mass of egg clutches, as hypothesized (Figure 4). We found three outliers at the points (1.8, 105), (5.8, 95), and (6.1, 85) using the same method as before (Figure 4). Figure 5 shows a random scatter, suggesting that the data is homoscedastic, as desired. Figure 5 also shows roughly equal variance and normality, since the standard deviation of the y-values for every fixed x is similar. Additionally, since the residuals follow an approximately normal pattern and the

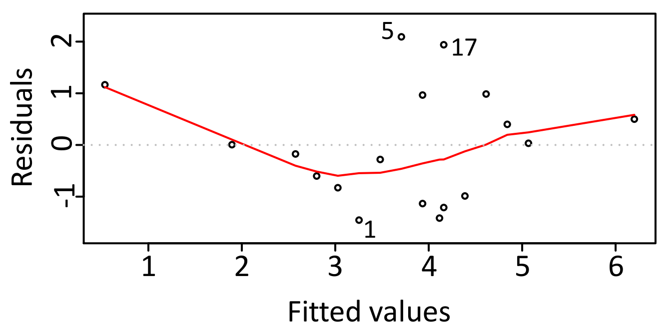

Figure 5. A scatterplot of the residuals for the 2018 data, which appear to be random, as desired.

distributions of the *y*-values for each *x* are approximately normal, we know that the sampling distribution of *b* is roughly normal.

We failed to reject the null hypothesis, $r = 0.754$, $b = -12.556$ grams per ppm, $n = 18$, $t(16) = -4.597$, $p < 0.001 < \alpha = 0.05$. We had strong that a negative linear relationship exists between chloride concentration and the mass of egg clutches in the spring of 2018.

Discussion

In our study, we attempted to determine whether chloride concentration negatively correlates to the mass of *Ambystoma maculatum* egg clutches. We hypothesized that strata in the vernal pools with higher average chloride concentrations would tend to have a lower average mass of *A. maculatum* egg clutches. Our results fail to support our hypothesis; however, historical data present strong evidence to support our hypothesis. Due to the conflicting results between the two sets of data, we cannot determine whether chloride concentration negatively correlates with the average mass of *A. maculatum*. A variety of factors may have affected our results, including insufficient and inaccurate data and egg mass conditions.

Our results are not consistent with those of past studies. Karraker and Gibbs (2011) found that after nine days, *A. maculatum* egg clutches in a 1 mg/L (low) chloride concentration had the highest average mass, while clutches in a 945 mg/L (high) concentration had the lowest average mass. As mentioned in the literature review, researchers determined the high concentration to be the maximum concentration they measured during 2002 and 2003 in roadside vernal pools located in the Adirondack region of New York, USA (Karraker et al., 2008). In our 2018 data, the chloride measurements ranged from 0.900 to 9.500 mg/L. In our 2019 data, measurements ranged from 0.000 to 2.800 mg/L. However, these measurements still fall far short of the 945 mg/L concentration which Karraker and Gibbs (2011) used as their high chloride concentration.

These relatively small amounts may be because the refuge most likely does not apply road salt on their roads; the chloride concentrations may instead be due to road salt which cars carry on their tires into the refuge after driving on salted roads.

In another study, embryo survival declined from 84% at a 1 mg/L concentration, to 68% at a 145 mg/L concentration, to just 3% at 945 mg/L concentration (Karraker et al., 2008). These results agree with those of Karraker and Ruthig (2009), where embryo survival rates at the time of hatching were 85%, 80%, 61%, 37%, and 7% in chloride concentrations of 1, 33, 145, 465, and 945 mg/L, respectively. The 2018 data results also displayed the trend of decreasing average egg mass as average chloride concentration increased within stratum from 1.700 to 6.700 mg/L. However, the results of the 2019 data displayed the opposite trend: average egg mass increased as average chloride concentration increased within stratum from 0.000 to 2.800 mg/L.

The ambiguity of our results may be due to female salamanders laying their eggs in their ideal conditions, known as oviposition preference. Oviposition preference may influence many survival factors including vegetation, concealment from predators, and water quality. In addition, certain clutches might simply have more eggs than others and consequently a larger mass. Due to combined factors like oviposition and egg mass variability, even if some strata had higher chloride concentrations than others, egg clutches within those strata may have larger average masses, conflicting with our hypothesis.

A lack of accurate data also contributed to our results' ambiguity. Our study initially required chloride measurements from every cell in every available pool; however, we had neither the time nor resources to do so. Instead, we randomly selected cells in each pool to collect water samples from and later measured the chloride concentrations from those samples. This random selection led to difficulties during our data analysis, as when we divided our pools into groups of cells, most strata had few or even no chloride measurements. In addition, while measuring chloride concentrations, we found multiple unlabeled samples, forcing us to eliminate those samples and further decreasing our sample size. Furthermore, the Vernier Chloride Ion Probes used to measure chloride concentrations may have malfunctioned during testing due to improper storage and usage, leading to possibly inaccurate measurements. Lastly, Highpoint II had only four egg clutches total, resulting in many strata without egg mass measurements.

Many researchers who conducted similar studies used laboratory environments, where they had plenty of resources and could create constants, reducing limitations. For example, Collins and Russell (2009) tested chloride solutions consisting of dechlorinated Halifax tap water and coarse food-grade salt (NaCl). In addition, Karraker and Gibbs (2011) made their solutions from road deicing salt (consisting mainly of NaCl) and dechlorinated tap water. In contrast to these two studies, we did not conduct our research in a laboratory environment. Because of this, we could not create chloride solutions to test the individual egg masses, as Karraker and Gibbs (2009) did. Additionally, Karraker et al. (2008) collected data by placing the egg clutches within a container filled with the desired concentration of road salt, which was placed afloat in the middle of the pool. Although they conducted their study in the field, they were able to control the amount of road salt concentration to observe the development of the egg clutches. We were not able to follow the same methods due to limited time and resources, and therefore could not control road salt concentrations.

For future studies, we would first recommend that researchers closely monitor their study site, which may reduce the impact of confounding variables. In particular, researchers should take two separate trips to their study site to allow for a matched-pairs *t*-test. Repeated measurements allow researchers to measure the development of specific egg masses over time.

Additionally, researchers should measure multiple explanatory variables, including conductivity and dissolved oxygen, to develop a multiple regression equation that can predict egg clutch mass based on various environmental factors. Lastly, to extend our study, researchers should study how road proximity affects embryo survival at multiple study sites. This research could show the negative effects of urbanization on salamanders and the environment.

To conclude, due to conflicting results between data collected in spring of 2018 and 2019, we cannot suggest a correlation between chloride concentrations and egg clutch mass. Our study had many technical difficulties which occurred during data collection and unforeseen confounding variables. To confidently determine if chloride concentrations negatively correlates with average egg clutch mass, researchers should conduct future experimental studies addressing the aforementioned limitations of our research and extending our research to road proximity and its impact on the environment. Since we could not find a correlation between chloride concentration and *Ambystoma maculatum* egg masses, we cannot determine if chloride concentration contributes to the amphibian decline.

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References

About the complex. (2013, May 20). Retrieved October 31, 2018, from U.S. Fish & Wildlife Service website: https://www.fws.gov/refuge/Mason_Neck/About_the_Complex.html

Blaustein, A. R., & Wake, D. B. (1990). Declining amphibian populations: A global phenomenon? *EcoTrends in Ecology & Evolution*, *5*(7), 203-204. doi: 10.1016/0169-5347(90)90129-2

Collins, S. J., & Russell, R. W. (2009). Toxicity of road salt to Nova Scotia amphibians. *Environmental Pollution*, *157*(1), 320-324. doi: 10.1016/j.envpol.2008.06.032

Corser, J. D. (2001). Decline of disjunct green salamander (*Aneides aeneus*) populations in the southern Appalachians. *Biological Conservation*, *97*(1), 119-126. doi: 10.1016/S0006-3207(00)00106-3

D'Amen, M., & Bombi, P. (2009). Global warming and biodiversity: Evidence of climate-linked amphibian declines in Italy. *Biological Conservation*, *142*(12), 3060-3067. doi: 10.1016/j.biocon.2009.08.004

Elizabeth Hartwell Mason Neck National Wildlife Refuge [Brochure]. (2011). Retrieved from https://www.fws.gov/uploadedFiles/Region_5/NWRS/South_Zone/Potomac_River_Complex/Mason_Neck/MasonNeckBrochure.pdf

[Elizabeth Hartwell Mason Neck National Wildlife Refuge to Washington, D.C.] [Map]. (n.d.). Retrieved from https://www.google.com/maps/dir/Elizabeth+Hartwell+Mason+Neck+National+Wildlife+Refuge,+High+Point,+Lorton,+VA+22079/Washington,+DC

Hossack, B. R., Smalling, K. L., Anderson, C. W., Preston, T. M., Cozzarelli, I. M., & Honeycutt, R. K. (2018). Effects of persistent energy-related brine contamination on amphibian abundance in national wildlife refuge wetlands. *Biological Conservation*, 228, 36-43. doi: 10.1016/j.biocon.2018.10.007

Karraker, N. E., & Gibbs, J. P. (2011). Road deicing salt irreversibly disrupts osmoregulation of salamander egg clutches. *Environmental Pollution*, *159*(3), 833-835. doi: 10.1016/j.envpol.2010.11.019

Karraker, N. E., Gibbs, J. P., & Vonesh, J. R. (2008). Impacts of road deicing salt on the demography of vernal pool-breeding amphibians. *Ecological Applications*, *18*(3), 724-734. doi:10.1890/07-1644.1

Karraker, N. E., & Ruthig, G. R. (2009). Effect of road deicing salt on the susceptibility of amphibian embryos to infection by water molds. *Environmental Research*, *109*(1), 40-45. doi:10.1016/j.envres.2008.09.001

Lips, K. R., Mendelson, J. R., III, Muñoz-Alonso, A., Canseco-Márquez, L., & Mulcahy, D. G. (2004). Amphibian population declines in montane southern Mexico: resurveys of historical localities. *Biological Conservation*, *119*(4), 555-564. doi:10.1016/ j.biocon.2004.01.017

Lowe, W. H. (2012). Climate change is linked to long-term decline in a stream salamander. *Biological Conservation*, *145*(1), 48-53. doi: 10.1016/j.biocon.2011.10.004

Muths, E., Corn, P. S., Pessier, A. P., & Green, D. E. (2003). Evidence for disease-related amphibian decline in Colorado. *Biological Conservation*, *110*(3), 357-365. doi: 10.1016/S0006-3207(02)00239-2

Sevin, J., & Kleopfer, J. D. (2015). Virginia's amphibians: Status, threats and conservation. *Virginia Journal of Science*, *66*(3), 27-307. doi: 10.25778/5fq1-ay82

Turtle, S. L. (2000). Embryonic survivorship of the spotted salamander (*Ambystoma maculatum*) in roadside and woodland vernal pools in southeastern New Hampshire. *Journal of Herpetology*, *34*(1), 60-67. doi:10.2307/1565239

Wildlife & habitat. (2013, May 14). Retrieved October 31, 2018, from U.S. Fish & Wildlife Service website: https://www.fws.gov/refuge/Mason_Neck/wildlife_and_habitat/index.html