The Effect of Road Salts ($MgCl_2$, NaCl) Concentration on Heartrate and Mortality in *Daphnia Magna* using Bioassay

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ABSTRACT

Road salts, commonly sodium chloride (NaCl) and magnesium chloride ($MgCl_2$), are frequently used for de-icing roads during the winter. However, these salts often find their way into watersheds and accumulate in lakes, increasing their salinity and pose a significant threat to aquatic ecosystems. Daphnia Magna, a species of zooplankton, are widely used as bioindicators in ecotoxicology studies because of their high sensitivity and transparent body structure. By subjecting Daphnia to 12 solutions varying in salt concentration (across $MgCl_2$ and NaCl) and recording their mortality and heartrate at four-time intervals, it becomes possible to establish salt concentration thresholds and the LD50 to determine the levels that cause significant harm to them and model ecosystems. The relationship between increasing concentrations of road salts and mortality rates was found to be statistically significant ($P=<0.01$), as well as the time in minutes and percent mortality ($P=0.05$). The concentration of each salt type also had a statistically significant relationship with the heart rate in beats per minute ($p=<0.01$). When examining the differences in salt types, there was no statistical significance for mortality ($P=0.76$) and heart rate ($P=0.46$). The LD50 for both salt types was found to be 1%. This study concluded that time in minutes, higher concentrations of $MgCl_2$, and higher concentrations of NaCl had increased mortality rates and decreased heart rates on daphnia, although no differences were found between the salt types.

INTRODUCTION

In the colder regions of North America, road salt application is a standard practice for maintaining road safety because of its low cost and availability (TRB 1991). However, the use of road salt has been increasing steadily over the past 60 years, commonly in the form of sodium chloride (NaCl) or magnesium chloride ($MgCl_2$) (Wiltse et al., 2020) (Findlay and Kelly, 2011). In 2014 alone, 24.2 million metric tons of sodium chloride was applied to roads in the United States (Lilek 2017). However, these salts often get swept into nearby watersheds, thus infiltrating the surrounding bodies of water with runoff ("How We Protect," n.d.). 37 % of the drainage area of the contiguous United States has experienced an increase in salinity over the past 50 years, citing road salt as the dominant source (Hinsdale, 2018). Small urban lakes receiving direct stormwater runoff are normally the most susceptible to the detrimental effects of road salt pollution (Dupuis et al. 2019). Lake Hortonia in Rutland County, Vermont, is a small, 479-acre, urban lake with a population of around 1,200 (Lake Hortonia, n.d.). Properties line the lake shoreline, many directly aside regularly salted roads such as Vermont Route 30 and 144. With 6 miles of shoreline and 7 square miles of drainage area, Lake Hortonia is highly susceptible to the dangers of road salt (Lake Hortonia, n.d.). An accumulation of excessive road salts can lead to increased salinity and a build-up that inhibits gas exchange with the overlying water (Hinsdale, 2018). Such conditions can result in oxygen depletion and cause anoxic conditions, harming the survival of aquatic organisms (Wiltse et al., 2020). The goal of this study was to assess the extent to which salinity is responsible for harming Daphnia Magna, to model whether the salinization of water resulting from saline inflows from traffic routes, like in Lake Hortonia, can be hazardous to aquatic biotopes. Daphnia magna, commonly known as water fleas, are widely used as bioindicators in ecotoxicity studies because of their high sensitivity, short life cycle, ease of culturing, and transparent body structure (Care Guide, n.d.). By conducting a bioassay subjecting Daphnia Magna to varying concentrations of NaCl and $MgCl_2$ and monitoring their responses in mortality and heartrate, it becomes possible to establish salt concentration thresholds and the LD50 (the % of salt that caused 50% of the daphnia to die) that cause significant harm. Results will showcase the effect that road salts have on daphnia and thus reflect outcomes on the entire aquatic ecosystem. This information can guide the development of guidelines and regulations for road salt application in places like Lake Hortonia to minimize future ecological damage.

Statement of Purpose

The purpose of this study was to determine the concentrations of $MgCl_2$ and NaCl that are harmful to Daphnia Magna to model whether the salinization of Lake Hortonia resulting from road salt inflows from traffic routes are hazardous to aquatic biotopes. Through mortality and heartrate, we established salt concentration thresholds and the LD50 that cause significant harm. This information aims to support guidelines and regulations for road salt application in Lake Hortonia to minimize future ecological damage.

Hypothesis

This study hypothesized that:

i) Increasing concentrations of road salts would increase daphnia magna mortality rates.

ii) Increasing concentrations of road salts would increase daphnia magna heart rates.

iii) NaCl would have higher heart rates and mortality rates than $MgCl_2$.

METHODOLOGY

Study Population

Experimentation was conducted in the science research laboratory at Saratoga Springs High School, in Saratoga Springs, New York, from October 2023 to November 2023. Daphnia Magna cultures were transferred from living in 3 separate shipment jars, around 30 adults per, to one collective tank 72 hours after arrival. The tank was filled with spring water and fed spirulina powder when needed, looking at water transparency for indication. Culture was stored in room temperature and dim-lit conditions.

Data Collection

Trials were done in two parts. Part one: mortality/mobility and part two: heart rate.

Part One: Six salinity solutions were created each for NaCl and $MgCl_2$ by diluting their 2% solutions. 2% solutions were made from 2g of Morton table salt without iodine (NaCl) or 2g of SafeStep rock salt ($MgCl_2$) mixed into 100ml of spring water. Six, 10ml dilutions of NaCl and $MgCl_2$ were made and put into test tubes: 2%, 1.5%, 1%, 0.5%, 0.2%, 0%. Using pipettes, five Daphnia Magna were added to each test tube. Mortality observations were recorded after 5, 10, 20, and 25 minutes. The surviving Daphnia Magna were transferred back into culture tank and remained fed and undisturbed for two weeks to reproduce and recover.

Part Two: Heartrate trials were performed two weeks after part 1 experimentation. The same twelve dilutions were remade using remade 2% solutions and poured into test tubes once again. One at a time, a single daphnia was transferred into the chosen dilution. After being left for five minutes, daphnia was transferred to concavity slide with pipette, removing excess dilution water to restrict movement. The slide was placed underneath a microscope at lowest brightness to limit heartrate interference. A recording iPhone captured daphnia heartbeats in slow-motion through the microscope lens for 15 seconds. Daphnia transferred back into same dilution and the process was repeated for the remaining time increments: 10, 20, and 25 minutes. Used daphnia safely disposed of with bleach upon completion. Part 2 experimentation was repeated with new daphnia for each of the 11 remaining dilutions.

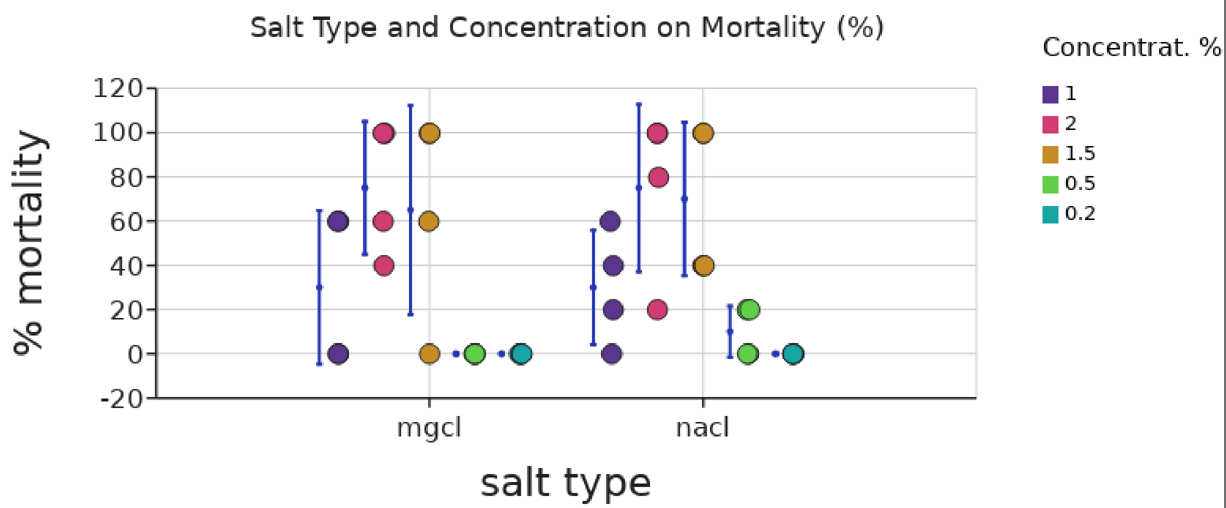
Statistical Analysis

Data is expressed as mean ± standard deviation. To analyze the quantitative data, Statistical Analysis Software (SAS) was used. The average heart rate of daphnia was taken from recorded heartbeats for 15 seconds and converted into beats per minute (BPM). The websites Data Classroom and Microsoft Excel were used to analyze and perform data analysis. A 2-way ANOVA test was used to evaluate salt type and concentration on mortality. The same test was used to evaluate salt type and time on mortality. Another 2-way ANOVA test evaluated salt type and concentration on heart rate. The LD50 for Daphnia in salt solutions were found to determine the % of salt that caused 50% of the Daphnia to die.

RESULTS

A statistically significant difference was found between the time in minutes and percent mortality with a p-value of 0.05 (Figure 2 and Table 2). Regarding the relationship between the concentration of each salt type and the percent mortality, a statistically significant difference was observed through the low p-value of <0.01 (Figure 1 and Table 1). The low p-value and relationship observed supports the first hypothesis that increasing concentrations of road salts would increase mortality rates and can be visualized in the strong positive relationship pictured in figures 4, 5, and 6. The concentration of each salt type also had a statistically significant relationship with the heart rate in beats per minute, seen with the p-value of <0.01 (Figure 3 and Table 3). However, this trend opposes the second hypothesis and shows increasing concentration has decreasing heart rates. Figures 7 and 8 visualize this inverse relationship. This study's third hypothesis could not be supported as a p-value of 0.46 was seen regarding the relationship between salt type and heart rates and a p-value of 0.76 was seen regarding the relationship between salt type and mortality (Tables 1 & 2). Therefore, it cannot be concluded that NaCl would have higher heart rates and mortality rates than $MgCl_2$. Finally, we can determine the LD50 for both salt types to be 1% (Figure 4).

Figure 1

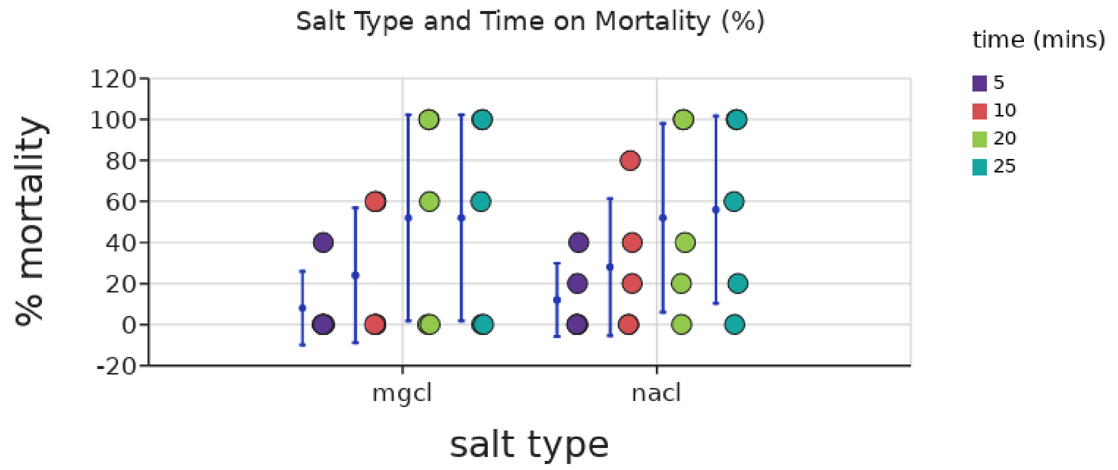


Note: Dot shows the mean. Error bars above and below show Standard deviation.

Table 1

Effect	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Squares (MS = SS/df)	F-statistic (MS / MS residual)	P-value	Interpretation of P
salt type (X)	90	1	90	0.12	0.74	Means: **no evidence** that there are differences between groups
Concentrat. % (Z)	38440	4	9610	12	<0.01	Means: **extremely strong confidence** that there are differences between groups
Interaction (X * Z)	160	4	40	0.052	0.99	Means: the effect of X does **NOT** depend on Z
Error or Residual	23300	30	776.7			

Figure 2

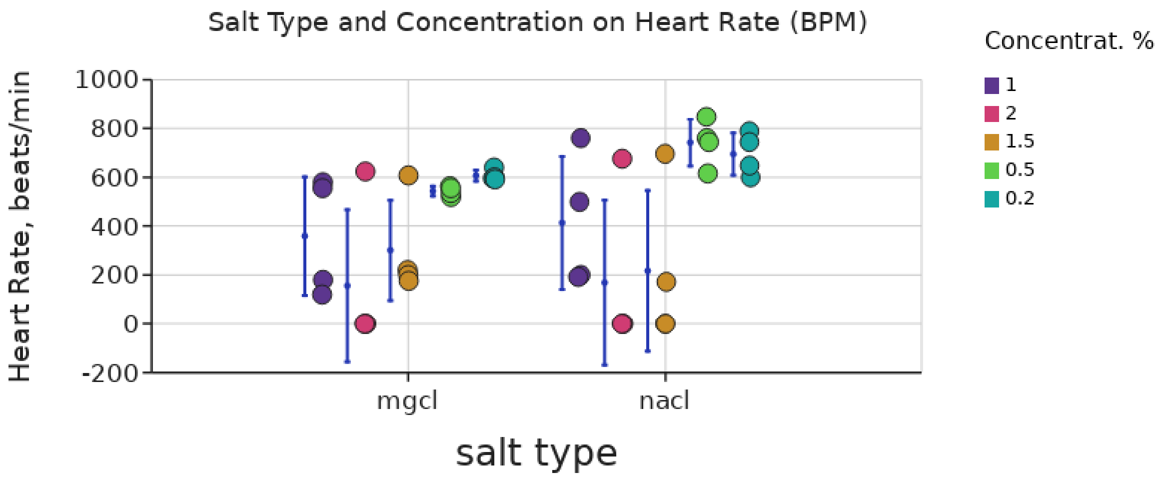


Note: Dot shows the mean. Error bars above and below show Standard deviation.

Table 2

Effect	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Squares (MS = SS/df)	F-statistic (MS / MS residual)	P-value	Interpretation of P
salt type (X)	90	1	90	0.06	0.81	Means: **no evidence** that there are differences between groups
time (mins) (Z)	13550	3	4517	3	0.05	Means: **strong confidence** that there are differences between groups
Interaction (X * Z)	30	3	10	0.0066	1.00	Means: the effect of X does **NOT** depend on Z
Error or Residual	48320	32	1510			

Figure 3



Note: Dot shows the mean. Error bars above and below show Standard deviation.

Table 3

Effect	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Squares (MS = SS/df)	F-statistic (MS / MS residual)	P-value	Interpretation of P
salt type (X)	28940	1	28940	0.56	0.46	Means: **little to no indication** that there are differences between groups
Concentrat. % (Z)	1572000	4	392900	7.7	<0.01	Means: **extremely strong confidence** that there are differences between groups
Interaction (X * Z)	85230	4	21310	0.42	0.80	Means: **no evidence** that the effect of X depends on Z
Error or Residual	1538000	30	51280			

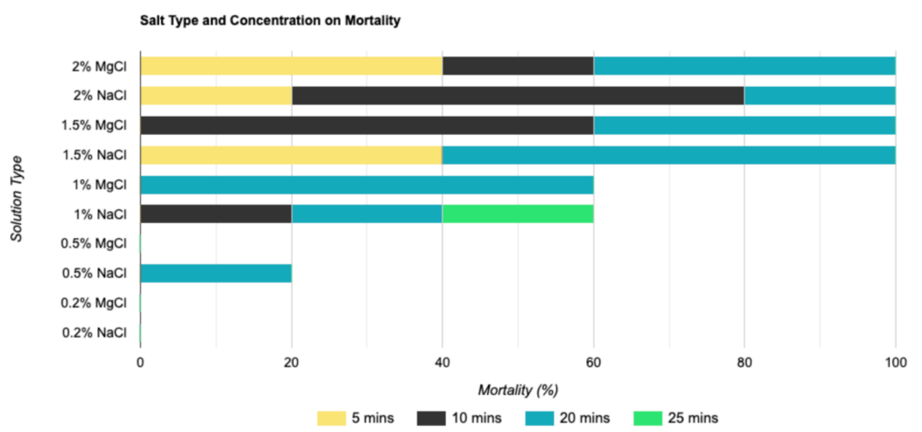


Figure 4

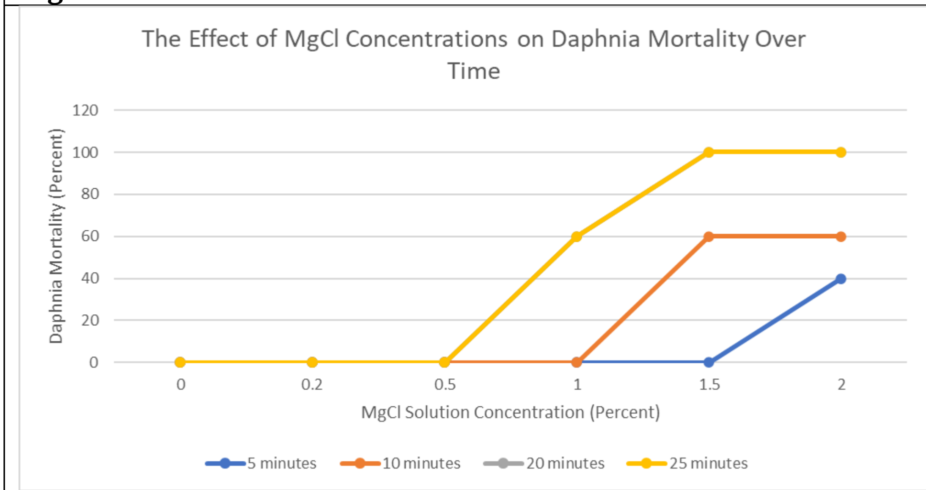


Figure 5

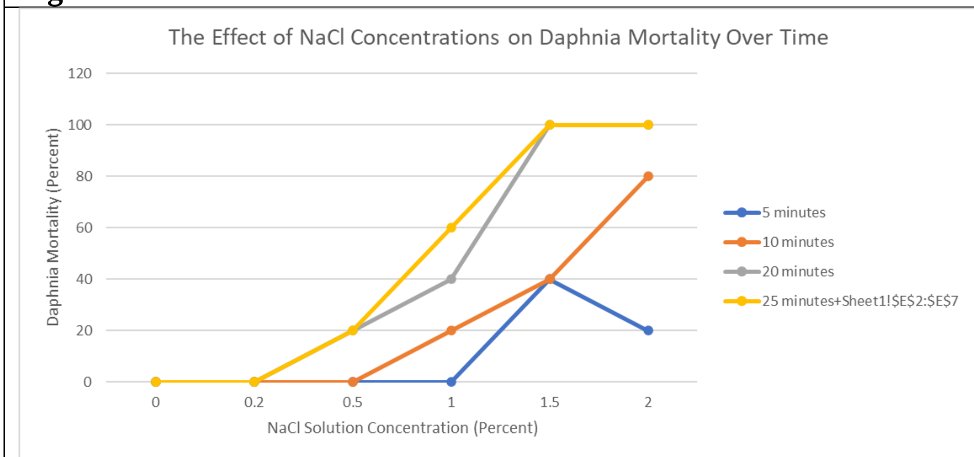


Figure 6

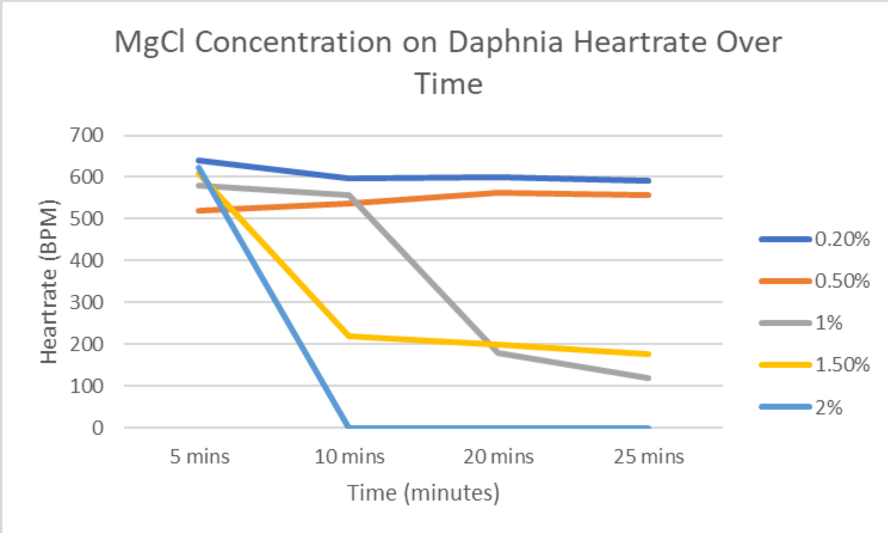


Figure 7

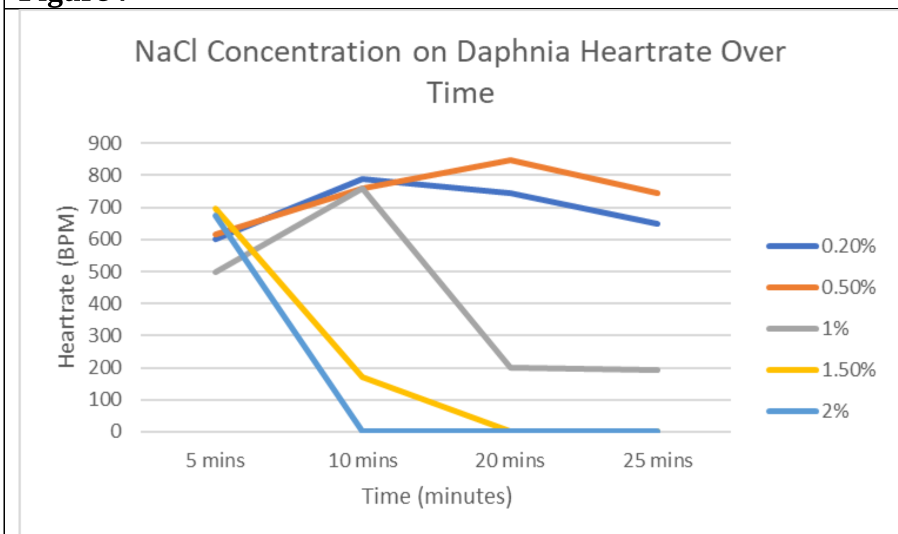


Figure 8

DISCUSSION

This study aimed to determine whether increased concentrations of road salts in water impacts the mortality rates and heart rates of Daphnia Magna, and whether the type of road salt ($MgCl_2$ or NaCl) causes a difference in responses. This research has concluded that increased salt concentration increases mortality rates and decreases heart rates. The decrease in heart rate opposes the second hypothesis, however, and could possibly be credited to Daphnia passing away, as many did not survive testing; Many heart rates initially increased but decreased on the way to mortality. There is no statistical significance found for salt-type-specific differences between $MgCl_2$ and NaCl in mortality rates and heart rates, although there was a data trend supporting higher heartrate peaks in NaCl. The limitations of this study include a small sample size of only five daphnia per dilution during mortality testing and one daphnia per dilution for heartrate testing.

CONCLUSION

The most significant result obtained from this study was that increased levels of $MgCl_2$ and NaCl are extremely harmful towards Daphnia Magna mortality rates and heart rates and is an issue that models the threat that environmental pollution poses to entire aquatic ecosystems as a whole. This research is intended to reflect the detriments of common road salt runoff in urban lakes, such as in Lake Hortonia, Vermont, and aims to aid in the solution. This study increases the knowledge of road salts' effects on the environment, specifically regarding mortalities, heartrates, and time. Additional research is needed to employ a larger sample size to produce more acute results and test more trials to investigate further impacts on heart rate and difference between salt types.

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