THE EFFECT OF SALINITY ON THE GROWTH RATE OF ZIZANIA PALUSTRIS IN CONTROLLED AND NATURAL SETTINGS

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Abstract: We compared the growth rate of wild rice, Zizania Palustris, in varying concentrations of road salt—0 mg/L, 100 mg/L, 500 mg/L, 1000 mg/L, 3000 mg/L, and 5000 mg/L. Then, we conducted two experiments, one in a controlled environment using a growth chamber, and the other outside. In the growth chamber, we had six replicates of each salt solution, giving us a total of 36 Petri dishes, with two seeds in each replicate. In the outdoor experiment, we set up three tubs and each contained nine plastic Solo® cups to grow the wild rice in. We put approximately 222 grams of sand from the shore of Douglas Lake in each cup and distributed the six solutions randomly throughout each tub. The remaining three cups contained only sand to make sure weight was distributed evenly and the cups had an equal amount of space within the tubs. We weighed the seeds in the growth chamber on day 5 of the experiment, and then weighed them again on day 7, along with the root and shoot of each plant. On day 10, we did the same for the plants outside. Using a significance level of 0.05 for both environments, we analyzed the seeds from the growth chamber and used a linear regression model. On both day 5 and day 7 in the growth chamber, weights of the wild rice plants confirmed that there was a negative relationship between salinity level and seed growth ($R^2 = 0.960$ for day 5 and 0.964 for day 7, both with a pvalue of 0.00). However, for the outdoor experiment, the linear regression test was not significant ($R^2 = 0.428$, p-value = 0.159). Both experiments showed significance when ANOVA tests were conducted (growth chamber p-value = 0.010, outside = 0.020) and we concluded that higher salt concentrations have a negative effect on the growth of wild rice. Using a Dunnett test we found this was true for the plants in the growth chamber, particularly at the higher levels of 3000 mg/L and 5000 mg/L.

INTRODUCTION

Rock salt (sodium chloride, NaCl) has become the de-icer of choice in the Great Lakes region during the winter months. In the 1940s and 1950s the "bare pavement" policy was adopted by highway agencies, and served as a standard for pavement conditions during severe weather. In order to have safe roads, they believed that "more is better" in terms of rock salt application (EPA 1999). According to the Highway De-icing report (NRC 1991), the average amount of rock salt used in the United States per year averages 8-12 million tons and when the snow and ice melt, the runoff of sodium chloride percolates into groundwater.

Elevated sodium chloride levels create an osmotic imbalance for various plant species, inhibiting water absorption, preventing root growth, and disrupting the uptake of nutrients (Wegner and Yaggi 2000). Sodium chloride inhibits root growth and germination rates of grasses and wildflowers at exposures as low as 100 ppm in soil (CEPA 2000). Other research shows that sodium chloride levels found in natural conditions can reach up to approximately 5,000 ppm (Garfield 2006).

Zizania palustris, a species of wild rice found in the Great Lakes region, is of great importance to the native people, the Anishinaabe, who use wild rice ("manoomin") as both a food source and in spiritual ceremonies. In addition, wild rice fields serve as habitats for many fish and wildlife where reproduction and foraging occur (MN DNR 2008). Z. palustris is found in areas of moving water such as rivers and inlets/outlets of lakes, growing well at depths of 0.15-.91 meters of water. An annual plant, it develops each spring from seeds that fall into the water during the previous autumn (MN DNR 2008). There are several factors such as seasonal water levels, lakebed conditions, climate, vegetation, and salinity levels that affect wild rice health and growth (MN DNR 2008). While plants require a certain amount of mineral salts to survive, high concentrations of mineral salts, including road salt, may be detrimental to the growth of wild rice. Z. palustris cannot grow in brackish water and is rarely found along sea coasts because salt concentrations are too high (Dore 1969).

Wild rice grows in very wet environments either completely or partially submerged in water (Garfield 2006). In the Great Lakes region, wild rice is on the decline, and in Michigan, it is a state threatened grass (Penskar et al. 2000). Over the past fifty years, its abundance has dwindled due to land-use practices and increased settlement which has changed the hydrological regime (Garfield 2006).

We were interested in observing the effects of various concentrations of rock salt on *Z. palustris* in order to determine if increasing salinity, due to increased road salt use, could affect the growth of wild rice. It has been proven that some rock salt concentrations will affect wild rice germination (Garfield 2006), but we were interested in determining in which salinity levels *Z. palustris* exhibited the highest and lowest growth rates. We chose to conduct a regression analysis in order to determine if a relationship existed between salinity levels and the growth rate of *Z. palustris*. In addition, we used an ANOVA test to ascertain if wild rice seeds grew at similar rates in different salinity concentrations.

METHODS

Zizania palustris in Outdoor Conditions

We used *Zizania palustris* seeds which were stored in a 35 degree Fahrenheit refrigerator since fall 2007, for wild rice seeds must be stored submerged in water at near-freezing temperature to maintain viability (Dore 1969). We placed the *Zizania palustris* seeds in a water-filled tray for 48 hours. After this time, we determined which seeds had begun germinating and then used these for our study.

We prepared five different concentrations of salt solution using a common road salt and water from Douglas Lake (Michigan, USA). We created salt solutions of 100mg/L, 500mg/L, 1000 mg/L, 3000 mg/L, and 5000mg/L in order to determine the growth rate of *Zizania palustris* in different salt concentrations and used a 0mg/L solution as our control. A previous study performed at Edgewood College in 2006 used similar measurements, but suggested that further research be conducted on higher salt concentrations. Therefore, we tested at 3000mg/L and 5000mg/L as well because in Garfield's study, the highest concentration found in natural conditions was 4560mg/L (2006). Then we put approximately 222.0 grams of natural sand

(found along the coast of Douglas Lake) into 24 plastic Solo® cups and made four replicates per solution. We then placed two germinated seeds in each cup along with 0.25L of each solution.

We randomly distributed the cups into three different plastic tubs filled with two liters of lake water in order to regulate temperature distribution. The three tubs were placed near the Shore of Douglas Lake in order to subject them to the natural conditions of Northern Michigan. We took conductivity readings on the fifth and tenth day of the experiment to observe any changes in salinity levels that might have occurred due to precipitation or evaporation. In addition, we recorded temperature and photometry readings on the fifth, seventh, and tenth day between the hours of 10:00am and 2:00pm in order to observe the environmental conditions that the wild rice seeds were exposed to. We chose this block of time because it has the greatest variation on an average day. Furthermore, we rotated the plastic cups every 48 hours within each tub to make sure they were exposed to similar conditions over the course of our 10-day experiment. To analyze our results, we weighed each root, shoot, and wild rice seed and performed an ANOVA and Linear Regression test at α =0.05 to determine if a relationship exists between salinity levels and growth rate of *Zizania palustris*.

Zizania palustris in Controlled Environment (Growth Chamber)

We used the same source of germinated seeds in order to test wild rice growth in a controlled environment. We made six replicates of each salt solution in Petri dishes, filled each dish to the same line with its respective solution and placed two germinated seeds into each dish. We then randomly arranged them on the same shelf within a growth chamber and observed their growth for seven days. Every 48 hours we rearranged the Petri dishes in order to randomize the experiment and expose each dish to the same conditions. The growth chamber was set at 19 degrees Celsius with 0% humidity, and had artificial lighting from 6:00am to 10:00pm every day. In addition, we took a photometer reading on the fifth day in order to determine the average

amount of light within the growth chamber. After the experiment was conducted, we weighed each root, shoot, and entire wild rice seed and performed an ANOVA and Linear Regression analysis at α =0.05

RESULTS

Linear Regression for Wild Rice in Growth Chamber

We found statistically significant evidence that illustrated a negative correlation between increased salinity levels and average seed weight (p=0.000, R^2 = 0.964, Fig.1). Therefore we rejected our null hypothesis and concluded that salinity levels affect the growth of wild rice seeds in the growth chamber.

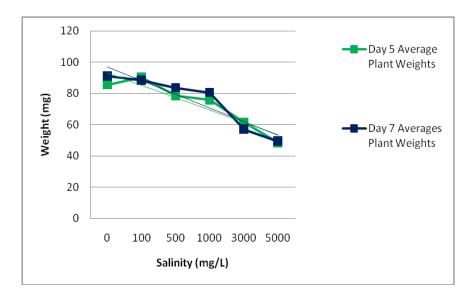


Figure 1. Linear Regression of Wild Rice in Growth Chamber (n=72, 6 replicates per solution). This graph illustrates the average wild rice weights grown in each salt solution on two different days. On both Day 5 and Day 7, the graph shows a negative correlation between increasing salinity levels and plant weight. For Day 5 $R^2 \!\!=\!\! 0.960$ and p-value of 0.000 and on Day 7 $R^2 \!\!=\!\! 0.964$ and p value of 0.000. Salt concentrations at or above 1000mg/L showed a pronounced decrease in plant weight in comparison to the 0mg/L, 100mg/L, and 500mg/L solutions.

ANOVA and Dunnett Analysis for Wild Rice in Growth Chamber

After finding a negative relationship between increased salinity concentrations and plant growth, we conducted an ANOVA test to determine if at least one of the salt solutions resulted in

different plant growth. The ANOVA test compounds the variances of all treatments to determine if the seed growth in all solutions is the same. On the fifth and seventh day, we found statistically significant evidence based on the average of our six replicates to conclude that at least one salt solution was different from the rest (Day 5 p=0.010; Day 7 p=0.020). However, ANOVA does not indicate which solution is different, so we proceeded to use a Dunnett test, which compares the mean seed weight of the control to the mean seed weight of each salt solution. On the fifth day, we found that the 5000mg/L solution was significantly different from the control (p=0.007). On the seventh day, we found that both the 3000mg/L and 5000mg/L solutions were significantly different from the control solution (3000mg/L p=0.042; 5000mg/L p=0.011). With this evidence, we can conclude wild rice growth in the 3000 mg/L and 5000mg/L saline solutions are significantly different than the control.

Root and Shoot Weights for Wild Rice in Growth Chamber

On day seven, we recorded the weights of the root and shoot of each seed separately and compared the averages (Fig. 2). This allowed us to observe which parts of the wild rice plant were most efficient at growing in different salinities. We found that a negative correlation existed, between both root and shoot growths and increased salinity levels (Fig. 2).

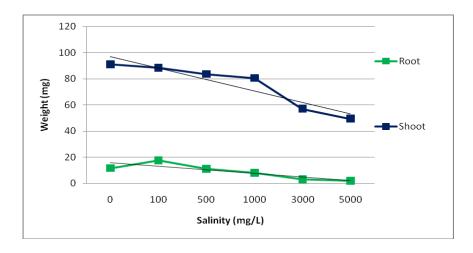


Figure 2. Comparison of Roots and Shoots in the Growth Chamber (n=72). This graph illustrates the average weight for the root and shoot of each plant. As salinity

levels increased, both the root and shoot weight decreased. However, they had a difference of 0.001 in their slopes. From this, we can postulate that the shoots were affected by increased salinity levels more so than the roots of the wild rice plant (Roots R^2 =0.768 and p-value 0.022; shoots R^2 =0.964 and p-value=0.00).

Linear Regression for Wild Rice in Outdoor Setting

Linear regression analysis of the total weights of the wild rice seeds grown outdoors shows a negative relationship with increased salinity (Fig. 3). However, the results were statistically insignificant ($R^2 = 0.428$, p = 0.159), but we believe that with more time, we could collect more data and have sufficient evidence to conclude that there is a significant relationship between increased salinity levels and wild rice growth.

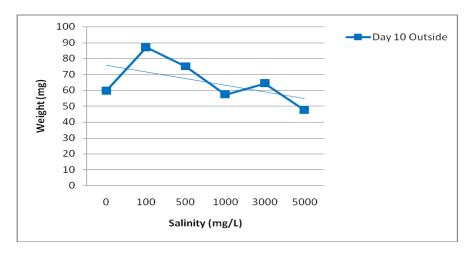


Figure 3. Linear Regression for Wild Rice in Outdoor Setting (n=48). This graph shows that there is a negative trend between plant weight and increasing salinity levels (R^2 =.428 and p-value 0.159). The average plant weight for the 100mg/L solution was higher than any other solution.

ANOVA and Dunnett Analysis for Wild Rice in Outdoor Setting

ANOVA analysis showed there was a significant difference in the growth of the plants among the solutions when they were compounded (p = 0.000). However, further analysis using a Dunnett test revealed that no solution caused plant growth to be significantly different from the control ($p_{100mg/L}$ =1.000, $p_{500mg/L}$ =.992, $p_{1000mg/L}$ =.772, $p_{3000mg/L}$ =.921, $p_{5000mg/L}$ =.431). Therefore,

while the ANOVA test was statistically significant, the Dunnett test failed to determine which particular solution resulted in decreased plant growth.

Linerar Regression for Root and Shoot Weights of Wild Rice in Outdoor Setting

A linear regression analysis comparing the weights of the roots and shoots of the seeds to varying salinity concentrations illustrates a negative relationship. Again, the results were statistically insignificant (roots: p = 0.155, $R^2 = 0.434$; shoots: p = 0.162, $R^2 = 0.423$), but we have consistently observed a negative relationship between increasing salinity levels and wild rice growth. Therefore, while we failed to reject the null hypothesis, we do not accept it.

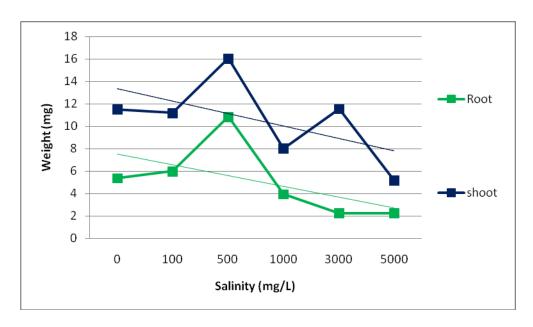


Figure 4. Weights of Roots and Shoots in Outdoor Setting. The overall trends shown in this graph for both root and shoot growth compared to salinity are negative, but are not statistically significant (Roots R^2 =0 .434 and p-value of 0 .155. Shoots R^2 =0.423 and p-value of 0.162).

Conductivity, Photometry, and Temperature readings in Outdoor Setting

In most cases, conductivity readings of the solutions decreased over time (Fig. 5). The decrease in salinity compared to the stock solution used in the growth chamber was likely caused

by precipitation and evaporation during the course of the experiment. Temperature and photometry readings taken of the wild rice outdoors were highly variable (Fig. 6).

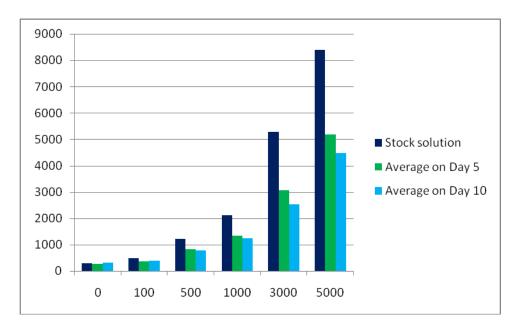


Figure 5. Average conductivities of solutions. The stock (starting) solution was used in both the growth chamber and the cups outside. Using a conductivity meter, we measured the conductivities of the four replicates and averaged them on days 5 and 10. There was a decrease in average conductivity over time in most solutions probably due to precipitation.

Date and Time	Bin	Temp in degrees Celsius	Photometer Outside (micromoles)
Day 5 11:30 am	Bin 1	16	09.03
	Bin 2	16	09.03
	Bin 3	17	09.03
Day 5 12:50 pm	Bin 1	17	285.8
	Bin 2	18	93.06
	Bin 3	19	1750.6
Day 7 10:55 am	Bin 1	12.5	224.3
	Bin 2	12	222.5
	Bin 3	13	209.2
Day 7 12:45 pm	Bin 1	13.5	223
	Bin 2	14	220.4
	Bin 3	15	221.4
Day 10 11:20 am	Bin 1	20	58.74
	Bin 2	20	193.3
	Bin 3	21	61.37
Day 10 1:05 pm	Bin 1	21	83
	Bin 2	21	217
	Bin 3	22	190

Figure 6. Temperature and Photometry Readings of Bin Water in Outdoor Setting. The temperature and photometry readings were not held constant throughout the outdoor experiment.

In conclusion, the results from the growth chamber clearly supported our hypothesis that increased salinity concentrations will have a negative effect on the growth rate of *Z. palustris*, while the results from the outdoor experiment were inconclusive. In the growth chamber, the environmental conditions were more consistent throughout the course of the experiment, but in the outdoor experiment, the salinity levels decreased probably due to precipitation.

Our results supported our hypothesis for the growth chamber. Wild rice seeds that were growth in less saline water were larger than ones grown in higher salinities in the absence of other environmental factors. The results of the experiment with seeds outdoors could not support our hypothesis, because we found no results that were statistically significant. However, the results from the growth chamber experiment and the slope of the regression line in the outdoor experiment suggest that our hypothesis still may be correct, but would require revisions to our methods.

The greatest factors that may have affected the outdoor experiment were the environmental conditions. The variability of the light and temperature may be the source of the poor R-squared value in our regressions for the outdoor experiment by making growing conditions less than ideal for the plants and making them grow more slowly. Additionally, the decreasing salinity of the solutions in the cups may have allowed the seeds to grow more successfully for each solution than would be predicted by the growth chamber experiment. Each solution was changed by a different proportion, which may have caused the weights of the plants to skew higher than what they might have been.

While the environment for the wild rice grown outside was closer to that which the plants would experience in nature, it was not representative of all the conditions they would experience. Rice grows in moving water at deeper depths than what our seedling were exposed to (Dore, 1969), while in our experiment the water was still and never replaced. Moving water may clear away pathogens or other organisms that were able to grow in the environment of the cups. The cups in our experiment had debris falling into them, especially insects, which would otherwise not have remained to affect the plants.

Fungal growth on the seeds seemed to be an indication of a lack of growth; all seeds that exhibited no growth were covered with fungus. If the lack of growth and presence of fungus can be taken to represent seed death, then our calculations include both the average growth of living seeds and the number of seeds that died during the experiment. However, to assess the growth of only living seeds, we would have to conduct the experiment with a much larger sample size because of the number of seeds that perished. In many of the solutions, half or more of the seeds died, leaving us with as few as two examples.

Likewise, an analysis of the proportion of shoot weight to root weight would have been interesting in illustrating the way the plants react to salinity, but there were not enough samples to give a reasonable analysis. Our regression analyses of the weights of the roots and shoots of the plants showed near-identical slopes for roots and shoots in each experiment; for the growth chamber the difference in slope was 0.001; for the outdoor experiment the difference was 0.0001. In both cases it was the shoot whose weight diminished more as salinity increased. This may imply that shoot growth is affected more by salinity, but a separate experiment would be needed to fully examine the relationship.

Whether the stress associated with osmotic imbalances causes the plant to emphasize the growth of either part, or if it has no effect at all has an important implication for the future life of the plant, because the shallow roots of the rice plant leave them vulnerable to blowing over in the wind (Dore 1969). If salt causes the roots to decrease in size, they would be further susceptible to wind damage.

Other de-icing compounds, such as calcium magnesium acetate, are becoming more popular as replacements for rock salt, although they are much more expensive (Vitaliano 1992). The effect of calcium magnesium acetate on wetland environments has been found to be far less damaging than road salt, as well as less corrosive to infrastructure (Fritzsche 1992), but little

research has been done on the effect of this compound on wild rice growth, and would be another line of inquiry for the future of both road safety and wild rice.

DISCUSSION

We hypothesized that *Z. palustris* seeds would grow less in higher salt concentrations because salt creates an osmotic imbalance in plants and inhibits water absorption, root growth, and the uptake of nutrients (Wegner and Yaggi 2000). Our hypothesis was supported for the plants grown in a controlled environment but not for plants grown in outdoor conditions. This may be explained by the precipitation the outdoor plants received which lead to a decrease in salinity. In the future, we should control for precipitation and evaporation by covering the outdoor cups to limit any fluctuations in salinity levels. Also, we did not see a noticeable difference between root and shoot growth for the outdoor plants as we saw in the growth chamber. This could be attributed to the cold temperatures the outdoor plants experienced. However, our study does show a negative correlation between salinity level and the growth rate of wild rice in a growth chamber. We specifically found that for concentrations of 3000mg/L and 5000mg/L of sodium chloride per solution affected the growth rate of wild rice. Therefore, we can report that wild rice exposed to these levels could further the plants decline and conservation plans should be implemented to help protect *Z. palustris*.

There were a few results and observations we found in our study that warrant further attention. For instance, in the growth chamber, we noticed the presence of fungus on the seeds. The fungus tended to grow mainly on the seeds in the higher salinity solutions. While we still feel confident the salinity levels were affecting growth rate, in future studies it would be ideal to control for this factor because it may have affected our recorded weights. The fungus may have grown because the water was stagnant and if we placed a small fan in the growth chamber we could have simulated moving water. The occurrence of a few rain storms during our experiment may have lead to the decreased salinity levels we observed with our conductivity readings (Fig

5). Since we attempted to replicate natural conditions for the outdoor plants, and we didn't see a negative correlation between plant growth and increasing salinity levels in the outdoor setting, it is possible natural settings may act as a buffering system. For instance, *Z. palustris* grows well in moving water systems like rivers, the inlets of lakes, and wetlands (MN DNR 2008). Therefore, in nature, wild rice may not be subjected to consistently high concentrations of salt because of changes in precipitation and the movement of water—unlike conditions in our experiment.

Conversely, since we didn't take conductivity readings for the growth chamber plants because our equipment lacked the capability to measure water levels less than an inch in depth, we cannot state that evaporation did not occur. Evaporation could have made the solutions more concentrated, which is why we could have seen more pronounced results.

Similar studies have observed the detrimental effects of increasing salinity levels on aquatics plants and animals. A study performed by Garfield (2006) noted the negative effect of salinity on the germination rates of *Z. palustris*. Garfield found levels exceeding 1000mg/L of chloride to be detrimental to wild rice. Another study conducted by Kaushal (2005) demonstrated that salinity levels exceeding 1000mg/L can be toxic to aquatic animals and can alter plant composition. Even levels as low as 250mg/L were found to be detrimental to freshwater plants and invertebrates. From this study, the researchers proposed that levels should not exceed 250mg/L of chloride as a standard for protecting aquatic life. Yet, Kaushal (2005) has been monitoring the salinity levels of North Eastern water bodies for the past thirty years and has observed that the salinity in streams, lakes, and rivers is accumulating. In Baltimore, salinity concentrations as high as 5000mg/L of chloride have been recorded during the winter months; this is equivalent to 25% the concentration of seawater (Kaushal et al 2005). They also noted a positive correlation between the increase of impervious surfaces and the increase in salinity levels of groundwater and surface waters—suggesting that as we create more roads, we use more

salt during the winter as a de-icer. Therefore, though nature has a built-in buffering system to decrease salinity levels, anthropogenic forces may be overriding this buffer.

With the findings of our study, it seems essential to consider management practices to promote the revitalization of this plant. One practice could be to preserve natural ecosystems like wetlands as a way to maintain the natural buffer system. Another suggestion could be proposing legislation to regulate road salt as a fresh water contaminate since it is an aquatic pollutant that currently is not being regulated (Kaushal et al 2005). Also, since it is difficult to prevent the development of new roads and the use of de-icers, we should examine the use of other de-icers like potassium formate as a substitute for sodium chloride (Nysten et al 2003). Further studies should investigate the use of other alternatives to sodium chloride and the possible impacts it could have on wild rice. Another possibility could be the process of phytoremediation to reduce the salinity content in contaminated water bodies. This process involves the use of salt tolerant plants to remove sodium chloride from water systems. However, the drawbacks to phytoremediation and the use of potassium formate is that it can be costly (Nysten et al 2003). Understanding the influence of salinity levels on the growth rate of Z. palustris can allow us to make predictions about the effect the rise in sodium chloride levels from road salt will have on wild rice and implement appropriate management practices to protect it.

We hypothesized that increased salinity levels would negatively affect the growth of the wild rice plant *Zizania palustris*. We thought that as the salt concentration of the water became higher, the plant's growth would decrease. A good portion of our results supported this hypothesis. For the growth chamber experiment we were able to completely reject the null hypothesis and conclude that increased salinity level causes a decrease in plant growth. While we weren't able to do the same for the outside experiment, we still do not accept the null hypothesis based our data. We did find a trend of increasing salt concentrations and decreased growth although it was not significant.

A previous study on the effects of road salt on wild rice hypothesized that higher concentrations would result in less seed germination. Solutions of 0 mg/L, 100 mg/L, 500 mg/L, and 1000 mg/L were used, which was similar to our experiment (Garfield 2006). This study was conducted inside a lab and is comparable to the controlled environment of our growth chamber experiment. The results provided conclusions similar to our own: the 0 mg/L solution had the highest average number of seeds germinated, 100 mg/L the second highest, and 500 mg/L tied with 1000 mg/L for the lowest number of seeds on average (Garfield 2006). Though the hypothesis was supported, the author did not think the concentrations used were ideal. For future studies she suggested solutions of 0 mg/L, 100 mg/L, 500 mg/L, 1000 mg/L, 3000 mg/L, and 5000 mg/L, which are the concentrations used in our study (Garfield 2006). Since our study and that of salt concentrations and seed germination had similar results, the conditions outdoors may explain why that experiment turned out differently than expected.

A possible explanation for the unexpected results we obtained from our outdoor experiment is the high frequency of rain during the course of our experiment. Using conductivity

measurements to estimate salinity levels, we concluded that the rain diluted the solutions so that their salt concentrations were less than the intended amount. We hypothesized that this decrease in salt levels is the reason our outdoor experiment data was not significant for two of the three statistics tests performed. This can be tested by performing another experiment in a controlled environment, such as the growth chamber. Without the inclement weather our salt solutions would not have become diluted. We would also need to make sure that no evaporation occurs, which would make the solutions stronger and also skew our results.

We found significant evidence that in a controlled environment higher salt concentrations result in lower growth rates for wild rice plants. In an uncontrolled environment other factors may interfere with the original salt concentrations and cause different results. Even with the dilution of our outside salt solutions we did find evidence that there were variances between the solutions, which shows that a change in salt concentrations does make a difference in the growth of wild rice plants. Whether or not the increased use of road salt will cause a significant problem for wild rice growth is uncertain. Without environmental factors the plants would certainly be in danger near salted roads, but with precipitation the concentrations can probably be lowered to a level where they are less harmful or not harmful at all. The salinity levels used in our experiment may be too high to be realistic. Salt levels close to 5000 mg/L have been reported (Garfield 2006), but are probably more of an exception than the norm. In order to obtain more conclusive evidence an experiment would need to be conducted in a completely natural setting using an existing wild rice field that is near a salted road and studying the salinity levels and their effects on rice growth.

We hypothesized that increased salinity concentrations would decrease *Z. palustris* growth and that at least one salt solution would differ in its ability to decrease wild rice growth. We were interested in this relationship because "the potential for road salt to contaminate groundwater has become a concern in several part of the country...where salt use is heavy" (MN DNR). Not only were we interested if a relationship existed, but also if wild rice can grow more successfully in certain saline concentrations than others.

In the outdoor setting, our ANOVA results indicated that there was a difference in the growth of *Z. palustris* in at least one out of the six salt solutions. However, we were unable to conclude which solutions had the greatest effect on decreasing the growth rate of wild rice. In the growth chamber, our results were statistically significant and we were able to conclude that there is a negative relationship between increased salinity levels and wild rice growth. We also were able to determine that wild rice growth exhibited significantly different growth in the 3000mg/L and 5000mg/L solutions by using a Dunnett test. This result supports Garfield's study which concluded that the highest levels of NaCl concentration had the lowest germination success rate (Garfield 2006).

While the experiment conducted in the outdoor setting was not as conclusive as the one in the growth chamber, we still found an overall negative relationship between increased salinity levels and wild rice growth in both environments. Concentrations as high as 5000mg/L have been found in natural conditions, so our findings have real-world applications and may be helpful in deterring such intensive use of road salt (Garfield 2006). However, we realize that the short duration of our study may have limited potential findings and if given more time, we may

have observed greater differences in growth rates and been able to gather more sufficient evidence to conclude what solutions wild rice grows best in.

Throughout our experiment, we found evidence of fungus on some of our wild rice plants and although we did not test this variable, we became interested in the implications that it might have had on wild rice growth and health. In addition, we took conductivity readings for the *Z. palustris* growing in the outdoor setting and found that salinity levels had decreased, most likely due to precipitation (Fig. 5). However, we did not run conductivity tests in the growth chamber and suggest that this be done in future studies to consider the possibility of evaporation occurring. Overall, our results from the growth chamber indicate that the higher salinity concentrations decrease wild rice growth. We encourage further investigation of this relationship and believe it would be useful to consider other factors such as environmental variation and the presence or absence of fungus on *Z. palustris*.

ACKNOWLEDGMENTS

We are especially grateful to Robert Vande Kopple, who helped us to build a fence for our outdoor experiment. Sherry Webster assisted us with our equipment and materials, and Michael Grant for the use of his chemistry lab and equipment. We also thank Robert Pillsbury and Sharon Shattuck for providing us with wild rice seeds, helping with the study design, and giving us overall guidance!

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