

Effects of Chloride Levels on Native and Invasive Aquatic Plants
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Abstract

Road salt (mostly sodium chloride) used as a winter de-icer on streets, sidewalks, and parking lots is known to negatively impact aquatic organisms as the salt is carried into lakes by rains and spring melts. This study compares salt tolerance levels of three species of aquatic plants, Eurasian milfoil (*Myriophyllum spicatum*), coontail (*Ceratophyllum demersum*), and lesser duckweed (*Lemna minor*). The purpose of this experiment was to determine whether the non-native species, *M. spicatum*, was more resistant to high salt levels than the native species, *C. demersum* and *L. minor*. Plants were exposed to a range of salt concentrations (100 mg/L; 500 mg/L; 1,000 mg/L; 5,000 mg/L; and 50,000 mg/L). Plant growth and reproduction were determined by measuring the length of plants and their respective levels of fragmentation. Increased salt levels resulted in a decline of growth and reproduction in *C. demersum* and *L. minor*, while levels of growth and reproduction in *M. spicatum* remained fairly constant at all salt levels. Algal density increased with increasing salt levels, suggesting high concentrations of salt may promote growth of algae at the expense of aquatic macrophytes, thus decreasing water clarity. This study supports other studies which show that road salt use in the Wingra watershed has detrimental impacts on aquatic ecosystems.

Introduction

The Wingra watershed has suffered negative effects of road salt use since the 1950's. High levels of chloride can lead to acute and/or chronic toxicity to organisms living in aquatic systems (SUS, 2006) * A report by the Salt Use Subcommittee in Madison states that "critical tolerance values of 10% of aquatic species in Madison lakes were exceeded for prolonged exposure to chloride concentrations (more than 220 mg/L)." The same report found evidence for a reduction in microinvertebrate diversity because of high chloride concentrations.

Salt is used during the winter as a road de-icer. When the rain comes in the spring, runoff carries the dissolved salt into the lake. Data recorded over the decades has shown a steady increase in chloride concentrations in Lake Wingra. Chloride concentration levels were 5 mg/l before the widespread use of road salt (Friends of Lake Wingra, 2003). The annual chloride level presented in the City of Madison Road Salt Report of 2005-2006 is 104.8 mg/L (Hausbeck, et al, 2006). These data suggest a severe increase in the use of road salt over the years. We wondered about the impact this had on Lake Wingra's organisms, particularly on aquatic vegetation.

Lake Wingra is a eutrophic lake, meaning that nutrients were added to it. This process occurred naturally through slow but steady erosion, runoff, and the importation of organic matter to the body of water by organisms. Cultural eutrophication occurs more rapidly as humans add nutrients to aquatic systems via the runoff of nitrogen-rich

* Salt Use Subcommittee. City of Madison.

fertilizers, wastes, phosphorous-rich detergents (Holmes, 2000), and road salt. Because of the constant nutrient input, the lake is susceptible to algae blooms. Algal explosions are followed by large amounts of decomposing algae, which cause oxygen depletion. But even under deteriorating conditions such as these, some species tend to thrive.

One such species is a macrophyte (aquatic plant) called *Myriophyllum spicatum*, or Eurasian milfoil. This submerged plant is an invasive species native to Europe, Asia and Northern Africa that was introduced into Lake Wingra in the early 1960's. About a decade later it had become the dominant submerged plant in the lake. Data from 1971 shows that the relative frequency of Eurasian milfoil was 68 percent. The other 32 percent was composed of many other native species, none of which had over 10 percent relative frequency (Davis et al., 1980).

M. spicatum is a very aggressive species. It reproduces quickly by fragmentation, creating a dense bed on the surface of the water, thus preventing other organisms from receiving sunlight. Researchers have found that *M. spicatum* can resist a variety of harsh environmental conditions. In New Zealand, it has been observed that *M. spicatum* can thrive in brackish water and in North America, it can survive Florida's high temperatures as well as overwinter in frozen lakes (Clayton, 2006). Because its stems and roots last through the winter, it grows rapidly in early spring, claiming the lake with a thick mat that overshadows other submerged plants. *M. spicatum* also inhibits human use of the lake for recreational purposes such as swimming and boating because decaying plants promote algal blooms.

The native macrophyte with the highest relative frequency of appearance, behind *M. spicatum*, is *Ceratophyllum demersum*. *C. demersum* has been described as a tolerant species that is usually dominant or subdominant (Davis, et al, 1980). It grows well in both oligotrophic (clear water lakes) and eutrophic environments. *C. demersum* prefers still waters because it does not have roots. Instead, it uses modified leaves at the base of its stem to anchor itself to the bottom. Davis, et al. (1980) reported that the species succumbed in Reelfoot Lake, Tennessee after heavy rains but came back strongly the following year. Lind and Cottam reported in 1969 that *C. demersum* in University Bay of Lake Mendota was found restricted to areas of low turbulence (Davis, et al, 1980). Those two instances are referred to as "short term" disturbances, usually one-time situations from which a species can recover. Intense eutrophication is a case of "long term" disturbance, which has affected many native submerged plants in Wisconsin. Lind et al. (1969) found that eutrophication since the 1920's had resulted in the decreased frequency of many pomatogens (like water celery), an increase of *C. demersum*, and a dramatic increase of *M. spicatum* (Davis et al., 1980).

We wanted to know whether *M. spicatum* was more salt tolerant than common native plants in Lake Wingra. We compared the invasive species to *C. demersum*, and to a floating vascular plant called *Lemna minor*. A separate study was set up to test chloride tolerance of *L. minor* because it is a different type of plant. *L. minor* is a diminutive plant often called "water lentil" or duckweed, because it is an essential component of some duck species' diet. It also reproduces by fragmentation, by growing lobes on its fronds that can separate and form new fronds (Cross, 2007).

From the information we obtained about *M. spicatum*, we hypothesized that the increasing chloride levels in Lake Wingra due to road salt use favors its dominance. The purpose of this experiment was to find the threshold of *M. spicatum*'s salinity resistance,

and to determine whether two other common native species are resistant to high chloride concentrations. In order to answer these questions we sampled *M. spicatum*, *C. demersum*, and *L. minor* and tested their respective salinity tolerances in microcosms. Our hypothesis was that *M. spicatum* grows better in high chloride levels than both *C. demersum* and *L. minor*.

Methods

We measured salinity tolerance of submerged plants, *M. spicatum* and *C. demersum*, separately from the floating species, *L. minor*. For the first two species, we prepared four solutions with different amounts of salt: 0 mg/l; 100 mg/l; 500 mg/l and 1000 mg/l. To make each solution, we poured 3 liters of deionized water into a jar, added 3.8 ml/l of hydroponic nutrient, and the corresponding amount of salt. This procedure was repeated three times to fill 16 jars, four replicates of each treatment. Each jar was labeled with the corresponding salt concentration and replication number.

For the *L. minor* experiment, we prepared a solution of 50 g of salt in 100 ml of tap water. Next, we used a serial dilution of the solution by a factor of ten, which means we added 10 ml of the solution plus 90 ml of tap water into another jar. We again diluted this last solution by a factor of ten into a third jar, and then repeated the procedure to produce a fourth jar of diluted solution. The contents of the first five jars are as follows: Control (0 mg/l); 50 g/l; 5 g/l; 500 mg/l and 50 mg/l. We set up four replications of each treatment for a total of 20 jars.

The next step was to gather plant samples. *M. spicatum* and *C. demersum* were collected from Lake Wingra, and *L. minor* from the Edgewood campus spring, on October 26, 2006. The first two species were cut into 20-cm long segments. A total of 16 fragments of each species were cut, and one fragment of each species was placed into each jar. Direct fluorescent light that covered the area evenly was placed about one meter above the 16 jars. Light was regulated by a timer that turned the light on from 6 A.M. to 8 P.M. Each jar was covered to avoid rapid evaporation. Algae blooms appeared in all jars of *M. spicatum* and *C. demersum* after a month, we believe because of water stagnation. To avoid higher rates of decomposition than were desired, we placed plastic pipes to pump air into the jars.

For the *L. minor* experiment, we sorted out one-hundred *L. minor* plants, each with four lobes, and put five plants in each jar. The jars were covered with plastic wrap and placed in an incubator kept at a constant 25 degrees Celsius.

All three species studied reproduce asexually by fragmentation. A plant “drops” fragments and from those, new plants can form. *M. spicatum* can also reproduce by seed germination, but this method is less frequent. The lobes in the periphery of *L. minor* plants separate to form new plants. Therefore, a single lobe, or unit, is considered a plant. We measured the reproduction of the three species under different salinity conditions by counting the number of fragments that formed over time.

The next variable measured was growth. We measured the length of the plants and compared them to the original 20 cm. The ability of the plant to grow and form a

thick mat cover was important for us to measure, since this is the habitat of invertebrates, fish, and other organisms in the lake.

Finally, we had not anticipated the algal blooms that appeared, and the role of algae in the experiment. Algal density can indicate organic pollution in water. So, we decided to measure algal density in each salinity level (only for *M. spicatum* and *C. demersum*) by counting the number of algae per ml. We collected water samples from each jar with a Pasteur plastic pipette. The pipette was placed in the center of the water surface until the 0.1 ml mark was reached, and then 0.1 ml of water was taken. The sample was then analyzed microscopically. Algae present in two traverses (two different locations in the cover slip) was counted, and algal genera were identified.

The Palmer equation below (Figure 1) is used for calculating a pollution index. We entered the genus and total number of individuals we obtained, and used the equation to figure out the level of organic pollution of water in each salinity level.

$$\frac{(\text{Area of cover slip}) \times (\text{number of individuals of each genus})}{(\text{Area of one traverse}) \times (\text{number of traverses}) \times (\text{volume under cover slip})}$$

Area of cover slip= 22mm x 22mm= 484 mm²
Diameter of field of 10 x objective lens= .95 mm
Area of one traverse= 22 mm x .95 mm
Area of traverse= 20.9 mm²

Fig 1. Equation for calculating Palmer Pollution Index

The Palmer equation gives an index to only a few genera, which are more commonly found in highly polluted waters. A second set of data was obtained by using an informal method, in which we identified and counted all the genera, and reported the totals (of each salinity level) as number of individuals per ml (see Appendix 1).

Results

The results show that the number of *L. minor* plants quadrupled over a month in the control jar, tripled at the 50 mg/l salinity level, and doubled at the 5 g/l level (Table 1). Duckweed did not tolerate the 50 g/l salt level. Plants in this jar died within two weeks and never reproduced (Table 1).

TABLE 1. Number of *Lemna minor* plants averaged from October 26 to November 27

Concentration	Number of Plants			
	October 26	November 6	November 15	November 27
Control Average	5	10.25	10.75	19
Control Standard Deviation	0	0.4	0.8	6.1
50mg/l Average	5	9.5	10	14.5
50 mg/L Standard Deviation	0	0.5	0.7	2.3
500mg/l Average	5	9.25	10.25	11.5
500 mg/L Standard Deviation	0	0.8	1.1	1.8
5,000mg/l Average	5	5.5	7.5	10.25
5,000 mg/L Standard Deviation	0	0.5	1.5	1.1
50,000 mg/l Average	5	4.75	0	0
50,000 mg/L Standard Deviation	0	0.5	0	0

Figure 2 shows an overall trend of reproductive decline.

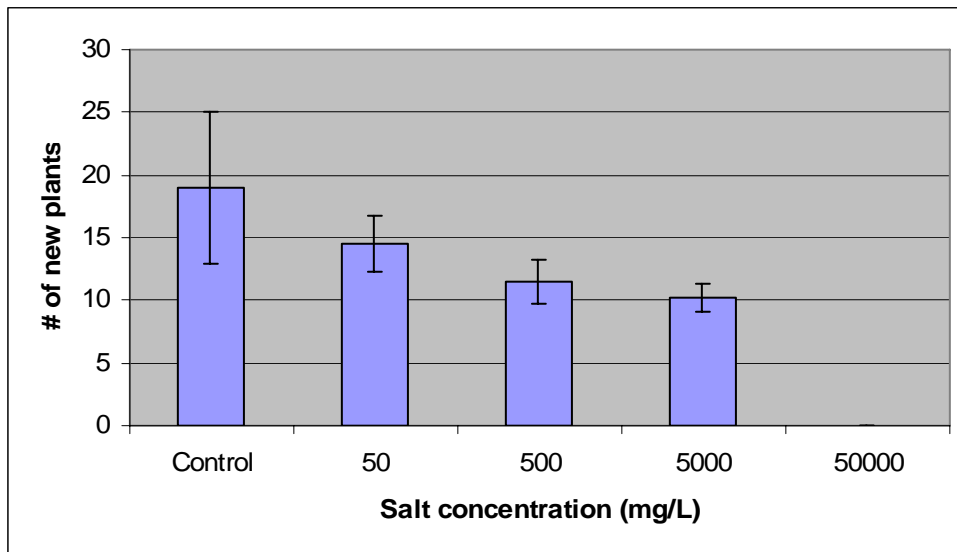


Figure 2. Effects of different salinity levels on *Lemna minor*'s reproduction by fragmentation. The y-axis indicates the number of new plants as of the end of the experiment. Bars indicate the standard deviation.

The results for *M. spicatum* and *C. demersum*'s reproduction by the second week of December are shown in Table 2. The right-hand column shows the number of fragments that were found in each jar. The level of fragmentation of *M. spicatum* is low in comparison to the other two species. However, its fragmentation level remained stable. These results suggest that the environment created for *M. spicatum* in this experiment might not have been the most favorable. However, the plants did not exhibit widely different reactions to increased salinity.

The level of fragmentation of *C. demersum* is much higher in the control jars than in any of the other jars (Table 2). The overall trend is that the level of fragmentation declines as the level of salinity raises. The overall trend for *C. demersum* is similar to that of *L. minor*.

TABLE 2. Length and number of fragments of macrophytes in different salt concentrations

Concentration	Eurasian Milfoil <i>Myriophyllum spicatum</i>		Coontail <i>Ceratophyllum demersum</i>	
	Total length (cm)	# of fragments	Total length (cm)	# of fragments
Control Average	34	5	197.8	12.5
Control Standard Deviation	4.2	4.2	1.8	6.4
100 mg/l Average	16.3	2	120.7	8.5
100 mg/L Standard Deviation	3.2	0	75.7	2.5
500 mg/l Average	22	2.6	41.6	6.3
500 mg/L Standard Deviation	20.3	2.1	5.1	0.6
1,000 mg/l Average	33.8	3.3	16.5	4
1,000 mg/L Standard Deviation	14.1	1.5	9	1.7

Figure 3 shows fragmentation levels of both *C. demersum* and *M. spicatum*. *C. demersum*'s level of fragmentation in the 1000mg/l jar was almost a third that of the level in the control jar.

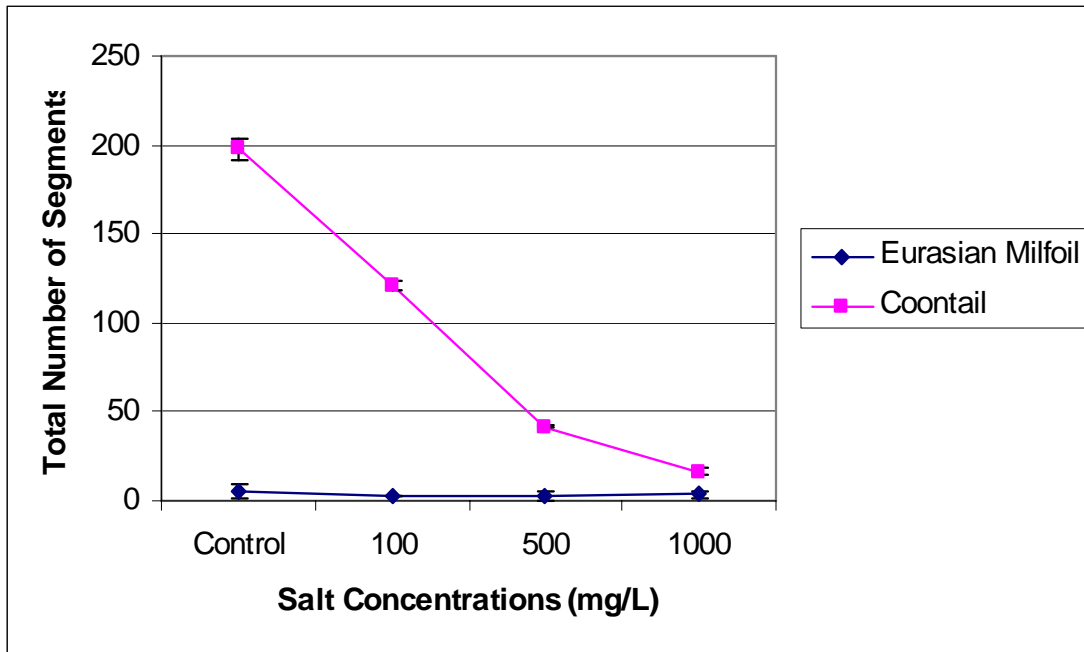


Figure 3. Levels of *C. demersum* and *M. spicatum*'s reproduction by fragmentation. Any segment equal or longer than 1 cm is considered a fragment.

Table 2 also shows plant growth. The left-hand column shows the results in centimeters (originally all plants were 20 centimeters long). *C. demersum* plants outgrew *M. spicatum* in the control jars and in the 100 mg/l concentration jars by an average of six times. However, as salt concentrations increased, the length of the fragments dropped dramatically. The results for *M. spicatum* show that although the plants did not grow as much as *C. demersum*, the length of the fragments remained constant at all salinity levels. The average lengths of the *M. spicatum* plants in the control jars are the same for the plants in the 1000 mg/l concentration jars.

Figure 4 illustrates how *C. demersum* outgrew *M. spicatum* in all but the higher salinity level, 1000 mg/L. As in Figure 3, the gap between species steadily narrows down as salt density increases. However, the results for plant growth show that *M. spicatum* begins outgrowing *C. demersum* as the latter approaches its salinity tolerance threshold.

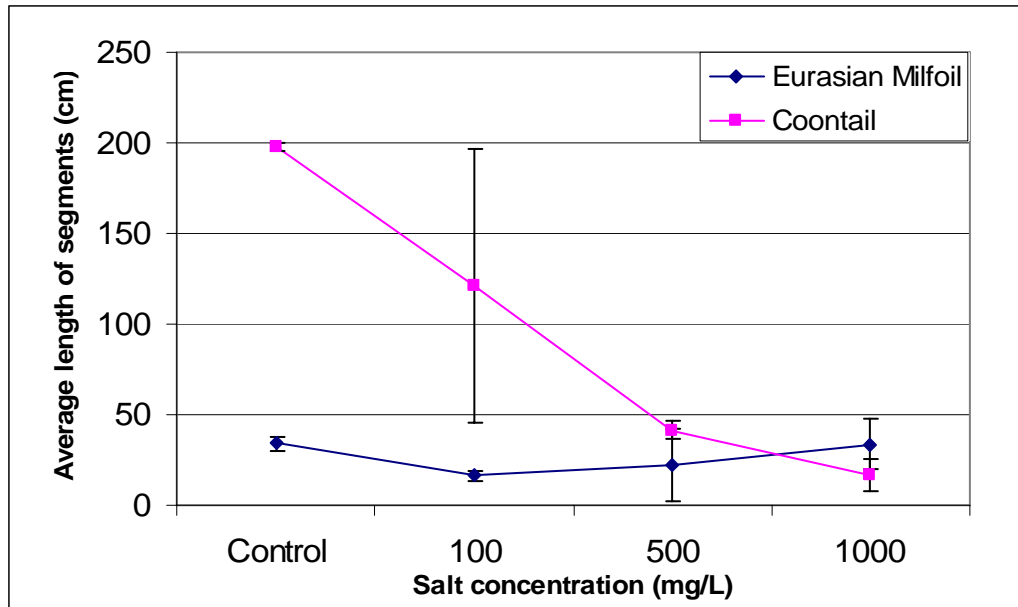


Figure 4. Overall trend of macrophyte growth in different salt concentrations.

The results of algal density calculations show an opposite trend of that of *C. demersum*. Algal production was found to increase as salinity levels increased. Appendix 1 shows all the algal genera identified. The shaded rows are genera used in the Palmer method. Table 3 shows a summary of the counts. The average algal density was calculated using the equation in the methods section, and appears in the far-right column.

TABLE 3. Algal density per ml using Palmer equation

Concentration	Algal genera	Density (algae per ml)
Control	Ankistrodesmus	695
	Synedra	116
	Stigeoclonium	116
	Scenedesmus	2200
Average Control		3127
100 mg/l	0	0
500 mg/l	Chorella	4400
1000 mg/l	Ankistrodesmus	13315
	Chlamydomonas	32421
Average 1000 mg/l		22868

Figure 5 shows algae's reaction to increased salt concentrations. The number of individuals (identified as most harmful by Palmer) per ml in the 1000 mg/L concentration is an average 19,741 more than the number of individuals per ml in the Control jars.

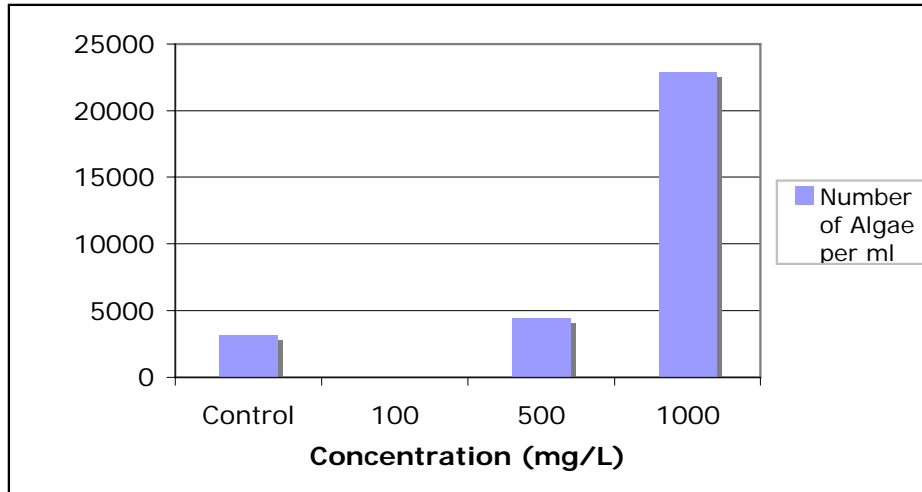


Figure 5. Algal density using Palmer equation (algae per ml)

Using the informal method (not using the Palmer equation), in which we counted the algae of all genera per 0.1ml, and then converted the results to algae per ml, we found similar results (Figure 6).

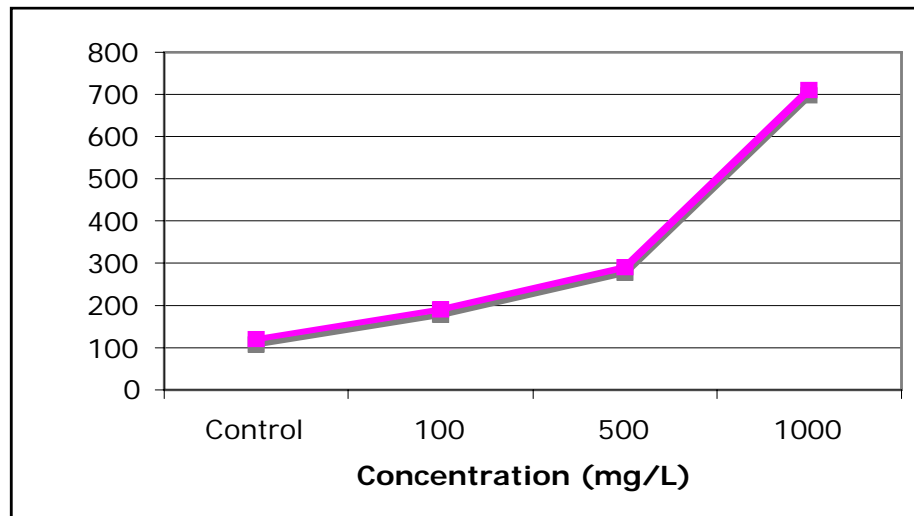


Figure 6. Algal density per ml, using informal method.

Both methods show a similar trend of algae growth, both of common genera and harmful genera found in organically polluted waters. No algae were detected or analyzed microscopically in the *L. minor* jars. Because of the size of the water sample (100 ml), most plants died sooner than the other two species. Also, judging by water color, there was no indication of abundant levels of algae developing in the treatments.

Discussion

Some researchers have found that *Myriophyllum spicatum*, an exotic species, can tolerate harsh conditions such as high temperatures and brackish water (Clayton, 2006). *Ceratophyllum demersum*, although a tolerant species, has a lower relative frequency than *M. spicatum* in Lake Wingra. Based on information gathered, we hypothesized that *M. spicatum* would fare better than the native species, *C. demersum*, in the higher salinity concentrations prepared for this experiment. *Lemna minor* is an abundant plant in Lake Wingra, and an important source of nutrients for waterfowl. We hypothesized increased salinity levels would be detrimental to this species as well.

The data we collected confirms our hypothesis in a general way. Some *C. demersum* plants in the 500 mg/l jars seemed to have grown more than *M. spicatum*; however, the overall trend demonstrated otherwise, that the rate of *C. demersum* growth was dropping dramatically while *M. spicatum* was surviving and reproducing at an almost constant rate in all solutions. Figure 3 shows that although *C. demersum* reproduces more than *M. spicatum* in every salt concentration, *C. demersum* undergoes a process of decline, unlike *M. spicatum*. As salt density increases, the gap between the two species narrows down. *L. minor* had similar reactions to high salinity concentrations than *C. demersum*. *L. minor*'s reproductive rate dropped steadily as salinity levels rose.

If we were to repeat this experiment, we would measure both plant length and reproduction of the three species more often. We would also try to find out what caused *M. spicatum*'s slow growth and reproduction rates. It is possible that under the better conditions for the plants, the results would have varied. In addition, other variables could have been measured before the plants started to decay, that includes weighing biomass of the aquatic plants and phytoplankton, and measuring water clarity.

Our data on algae suggests that high salinity concentrations contribute to high algal density. The results for *L. minor* show that as salt concentrations in water increase, reproductive potential of *L. minor* declines. *L. minor* tolerates salt and can reproduce when there are up to 5 grams of salt per liter of water. However, reproduction rates are much higher for individuals in the control jars. We believe the increased algal density in the water may have contributed to the decay patterns observed in the macrophytes as well. In spite of having used two different methods to calculate algae density, our identification of species may contain a high degree of inaccuracy. Also, the algae counts may be arbitrary considering phytoplankton's size and extreme abundance in some samples.

The documented effects of high levels of chloride on macrophytes can have practical implications. First of all, there is established evidence that phosphorus and nitrogen levels are rising in the lake and contributing to further eutrophication. More data are necessary concerning the role of chloride in eutrophication, and what that means for native species of macrophytes (Byler, et al, 1999). An extension of this experiment could center on more native species like Potamogetons, which have a lower frequency of appearance in the lake than *C. demersum*.

If cultural eutrophication continues at the current pace, chloride is likely to increase to unmanageable levels. Using salt to de-ice roads and nutrient loading pose threats to the Lake Wingra ecosystem. First, as the experiment shows, high chloride levels contribute to algal blooms and increased algal density. Subsequent algal decay in

great quantities puts the ecosystem at risk of oxygen depletion. Second, on our experiment, the high salinity in water did not have serious negative effects on the dominant, exotic macrophyte species. We concluded that high chloride concentrations promote conditions that favor the invasive species, and put native species at a disadvantage.

The dominance of *M. spicatum* needs to be addressed because it contributes to the overall trend of reduction in species diversity in the lake (Davis et al., 1980). Many species of pondweeds, for instance, which are essential food for some waterfowl, are increasingly harder to find. *C. demersum*, the second most frequent macrophyte, also provides a good source of nutrients for some species of diving ducks that do not eat *M. spicatum* in great amounts (Kenow, et al, 1985). *C. demersum* accounts for almost 60 percent of laying Redhead ducks, and *L. minor* constitutes almost 60 percent of the diet of pre-laying Redheads. *C. demersum* is also a habitat to many invertebrates that are an essential source of protein for some organisms. Increased chloride, which is detrimental to *C. demersum*, will affect invertebrates as well. In order not to further disrupt food chains, it is important to reduce chloride levels that harm aquatic species, invertebrates, which live in the dense beds of *C. demersum*, and waterfowl diversity.

Recommendations for further study include:

- Finding thresholds of salinity tolerance for other aquatic species, like pondweeds. Many duck species in Lake Wingra consume Water Celery in great percentages; therefore, we recommend a study on salinity and Water Celery.
- Identifying various species' tolerance to low levels of oxygen that may be affected by algal blooms, promoted by increased chloride levels in the lake.
- Studying the effects of increased salinity on invertebrates associated with *C. demersum*.

Appendix Section

Appendix 1. Algae counts

CONTROL 1	Algal Genera	Total algae	Palmer Index
	Colonial	10	0
	Ankistrodesmus	6	2
	Cladophora	21	0
	Synedra	1	2
	Stigeoclonium	1	2
	Unicellular	26	0
100 mg-	Colonial	6	0
	Unicellular	42	0
500 mg-1	Colonial	6	0
	Unicellular	58	0
	Placoderm desmids	14	0
1000 mg-1	Colonial	26	0
	Bulbochaete	1	0
	Tabellaria (Diatom)	2	0
	Ankistrodesmus	115	2
CONTROL 2	Colonial	8	0
	Unicellular	0	0
	Placoderm desmids	42	0
	Scenedesmus quadricauda	19	4
	Filamentous	1	0
100 mg-2	Bulbochaete	2	0
	Placoderm desmids	24	0
500 mg-2	Chlorella	38	3
1000 mg-2	Chlamydomonas	280	4
	Microcystis colony	3	0

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