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Title of Thesis: THE ROLE OF ROAD SALT DEICER AND RESOURCE BIODIVERSITY ON AQUATIC CONSUMER FEEDING DYNAMICS

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ABSTRACT

Title of Document: THE ROLE OF ROAD SALT DEICER AND RESOURCE BIODIVERSITY ON AQUATIC CONSUMER FEEDING DYNAMICS

Lauren Nicole McDonald, Master of Science, 2021

Directed By: Professor, Christopher Swan, Geography and Environmental Systems

Road salt deicer is a growing pollutant of concern in the United States as urbanization and human expansion continues at a rapid rate. Deicer infiltrates surface waters through stormwater runoff and increases chloride concentrations in freshwater ecosystems. The purpose of this study was to evaluate the effects of road deicer on a critical function of freshwater ecosystems - leaf litter breakdown. I hypothesized that increased chloride concentrations would decrease breakdown rates through decreased invertebrate consumer activity. To examine the impacts of chloride breakdown rates, a multi-factorial laboratory experiment was designed to test the effect of chloride on aquatic consumer feeding rates and examine the role of heterogeneous leaf litter under these conditions. Leaf litter and aquatic invertebrates (*Gammarus sp.*) were collected from headwater streams in

Maryland (USA). Microcosms were inoculated with stream water and subjected to a chloride treatment (0 or 600mg/L), a leaf litter treatment (*F. grandifolia*, *A. rubrum*, or "mixed"), and an invertebrate treatment (presence or absence). After 28 days, mass loss rate was calculated for all treatments. In the single-species litter treatments, mass loss increased under the presence of chloride in both the presence and absence of the invertebrate consumer. Regardless of invertebrate presence, chloride only decreased mass loss in the mixed leaf litter assemblages. Further, the results showed a diversity effect between the single and mixed-species litter treatments but only in the invertebrate absent treatments. Therefore, the presence of an invertebrate eliminated the diversity effect regardless of chloride concentration. In short, increased chloride concentrations altered feeding rates though differently than predicted while a diversity effect was observed only in the absence of the invertebrate. The results suggest a complex interaction between chloride, heterogeneous leaf litter, and aquatic consumers that requires additional research to further understand the relationship between these factors.

KEYWORDS

Chloride | Freshwater streams | Invertebrate consumers

Leaf litter breakdown | Road deicer

THE ROLE OF ROAD SALT DEICER AND RESOURCE BIODIVERSITY ON AQUATIC CONSUMER FEEDING DYNAMICS

By

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INTRODUCTION

In freshwater ecosystems, allochthonous inputs of leaf litter serve as the primary source for carbon processing. Leaf litter breakdown in freshwater systems is a well-studied process from the physical leaching of nutrients to the chemical breakdown by microbes and invertebrates (Peterson & Cummins, 1974; Wallace et al., 1982; Webster & Benfield, 1986). Most organic matter decomposition occurs in the headwater streams and is heavily influenced by abiotic factors such as temperature, acidity, and water chemistry (Melillo et al., 1982).

Increased urbanization is altering ecological processes at large spatial scales (Kaushal et al., 2018). Urbanization results in changes to land use and land cover that includes increased impervious surface cover, decreased infiltration, and increased surface runoff (Bird et al., 2018; Brown et al., 2009). During winter months, road deicer is applied to roadways but following application much of the deicer washes off the roads and into nearby streams (Daley et al., 2009; Lax et al., 2017). Research suggests that road deicer usage can reduce vehicle crashes and prevent road related injuries (Fu & Usman, 2013) and, as such, an estimated 20 million tons of road deicer is applied to roadways across the United States every year (Kelly et al., 2019). As demand for road deicer increases simultaneously with human growth and urbanization, runoff of road deicer into surface waters will also increase (Kaushal et al., 2018). All of these factors have resulted in increased salinization in freshwater ecosystems particularly in receiving headwater streams (Daley et al., 2009; Kaushal et al., 2005; Morgan el al., 2012).

Road deicer usage varies regionally, largely correlates with winter precipitation, and few regulations exist nationally to prevent and reduce its usage (Kelly et al., 2010, 2019). In Baltimore, Maryland (USA) chloride concentrations have been found to exceed 250 mg/L in many urban streams, with concentrations over 3000 mg/L at times (Kaushal et al., 2005; Stranko et al., 2013). According to research by Blasius and Merritt (2002), invertebrate species were affected by chloride concentrations as low as 240 mg/L while some tolerated concentrations up to 800 mg/L, measured by mortality rates. Chloride concentrations in headwater streams are variable, increasing sharply following road deicer application in the winter months (also referred to as episodic salinization or pulsed increases in concentrations) and decreasing to low, stable levels during warmer summer months (Haq et al., 2018; Kaushal et al., 2005). Research suggests that both acute and long-term exposure to increased chloride levels can have impacts on various aquatic species (Allert et al., 2012; Cañedo-Arguelles et al., 2013; Swan & DePalma, 2012; Van Meter et al., 2012).

Invertebrates play a key role in the decomposition of leaf litter in freshwater ecosystems; therefore, impacts to the invertebrates can alter the breakdown process (Swan & DePalma, 2012). In addition to aquatic consumers, chemistry itself plays an important role in the breakdown process. Freshwater ecosystems are detritus-based ecosystems that rely on leaf litter inputs from vegetation, also known as allochthonous leaf litter inputs. The presence of secondary chemicals in leaf litter contributes to varying breakdown rates of leaf litter species (Lecerf & Chauvet, 2008; Ostrofsky, 1997; Peterson & Cummins, 1974). Higher concentrations of these secondary chemicals (tannins, lignins, etc.) and lower levels of essential nutrients (N, P, etc.) slow overall decomposition rates (Melillo et al., 1982) and are generally less favorable by aquatic consumers (Swan & Palmer, 2006b).

Diversity of leaf litter also drives breakdown rates of organic matter (Lecerf et al., 2011; Swan et al., 2009; Swan & Palmer, 2004). The effects of leaf litter diversity on aquatic ecosystems cannot always be explained by summing the individual contributions of each leaf litter species (Gessner et al., 2010). This phenomenon is called the diversity effect and additional research suggests invertebrates can influence diversity effects (Gessner et al., 2010). Additionally, aquatic consumers exhibit preferential feeding of detritus resources and can increase feeding rates on low essential nutrient litter in order to obtain the necessary nutrients, called compensatory feeding (Gessner et al., 2010; Swan & Palmer, 2006b). Thus, diverse leaf litter assemblages reduce variation in breakdown rates and provide more stability in the organic matter breakdown rates compared to single-species leaf litter assemblages (Swan & Palmer, 2006a, 2006b). These findings occurred under controlled aquatic environments representing healthy stream conditions. As urbanization continues, fewer healthy stream conditions persist and the influence of environmental stressors increases. Stress can alter ecosystem processes (Cañedo-Arguelles et al., 2013; Hintz & Relyea, 2017; Swan & DePalma, 2012) but the role of resource biodiversity remains largely inconclusive (Fugère et al., 2012).

The objective of this research was to evaluate 1) if the presence of road deicer, referred to as chloride in this paper, alters feeding rates of aquatic consumers (*Gammarus sp.*), 2) if heterogeneous leaf litter alters aquatic consumer (*Gammarus sp.*) feeding rates under the presence of a stressor, 3) whether there are any interactions between these two factors. The approach consisted of a multi-factorial microcosm experiment carried out in a laboratory setting which allowed for the most control of potential confounding variables. Using evidence from previous studies (Morgan et al., 2012; Swan & DePalma, 2012), it was predicted that aquatic consumers would have a lower feeding rate, expressed as less mass loss, under the presence of chloride. Elevated chloride levels as a result of increased salinization can alter water chemistry, disrupt metabolic processes, induce stress on aquatic consumers, and negatively influence carbon processing in freshwater ecosystems (Allert et al., 2012; Cañedo-Arguelles et al., 2013; Stranko et. al., 2013; Swan & DePalma, 2012) Response to chloride stress has been well studied, but few have examined the potential ability to mitigate the effects through increasing leaf litter diversity.

MATERIALS AND METHODS

Experimental Design

A microcosm study design was selected to evaluate changes in *Gammarus sp.* feeding rates under the presence of elevated chloride levels. The microcosm design allowed for controlled chloride levels (0 and 600 mg/L) throughout the duration of the study. Chloride levels were selected based on previous research in the mid-Atlantic region (Kaushal et al., 2005; Swan & DePalma, 2012). Specifically, 600 mg/L was selected because it is above 250 mg/L, which is often considered the baseline level for freshwater toxicity, but below the threshold for high levels of invertebrate mortality which is often seen at concentrations over 800-1000 mg/L (Blasius and Merritt, 2002). Homogeneous and heterogeneous leaf litter treatments were also included to test the effectiveness of a mixed leaf litter assemblage on mitigating the impact of an environmental stressor.

In Fall of 2019, leaf litter was collected directly following abscission from individuals of *Fagus grandifolia* (American Beech) and *Acer rubrum* (Red Maple) located adjacent to the Patapsco River in Howard County, Maryland (USA). The Patapsco River watershed lies within the Piedmont region of Maryland. The riparian communities are dominated by *Fagus grandifolia, Quercus alba, Acer rubrum,* and *Platanus occidentalis* (Swan et al., 2009). *Fagus grandifolia* and *Acer rubrum* were chosen over other leaf litter species because they are more likely to dominate the litter pool in late winter and early spring when deicer levels are higher. Following collection, leaves were taken back to the lab where they were stored and air dried in paper bags. Leaves were submerged in water, cut into 2.5 cm diameter leaf disks, and air dried. Once dry, leaf disks were dry massed and placed into mesh bags measuring approximately 20cm x 10cm with a mesh size of 1.5mm. Each mesh bag contained six leaf disks of either species depending on the treatment. The “mixed” leaf litter treatment contained three *F. grandifolia* leaf disks and three *A. rubrum* leaf disks.

The leaf shredding amphipod, *Gammarus sp.*, is a common species in the Piedmont region known to tolerate degraded water quality like that seen in urban drainage systems such as the Patapsco River (Swan & DePalma, 2012). In early February 2021, amphipods were collected from a tributary of the Patapsco River located in Patapsco State Park in Howard County, Maryland. After collection, individuals were stored in a lab for five days in aerated stream water and allowed to feed on leaf litter material collected at the time of sampling.

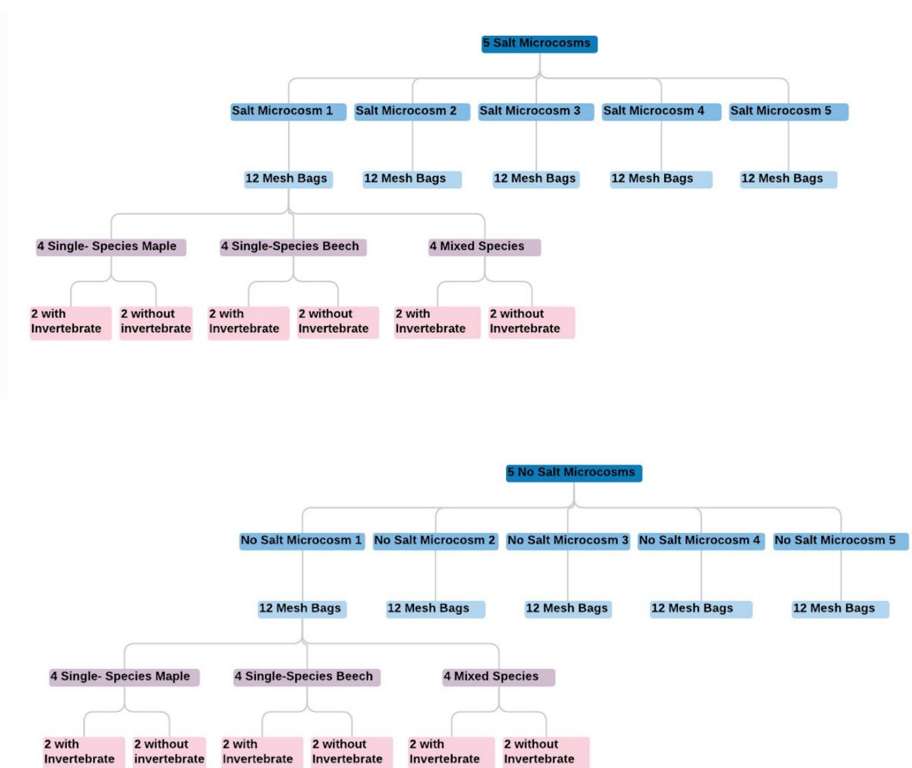
Ten microcosms were created using plastic bins (15.75” x 12.5” x 6”) on a laboratory workbench. Each microcosm consisted of a plastic bin filled with 7 L of filtered (45 um) stream water from the same tributary of the Patapsco River where the amphipods were collected. Each microcosm was randomly assigned a chloride treatment (0 or 600 mg/L), leaf litter treatment (*F. grandifolia*, *A. rubrum*, or "mixed"), and invertebrate treatment (presence or absence) (Figure 1). Once treatments were assigned, the microcosms with the chloride treatment were inoculated with 6923.77 mg of sodium chloride (NaCl). Twelve leaf litter bags were then randomly assigned and added to each microcosm based on the assigned treatment group (Figure 1).

Microcosms were left undisturbed for 48 hours to allow for pre-conditioning and leaching of the leaf litter. After 48 hours, two amphipods were added to each leaf litter bag in the amphipod-presence assigned microcosms using a transfer pipette. The experiment began on February 15, 2021 and ran for 28 days. After the first 7 days, each amphipod-presence assigned microcosm was inspected for amphipod mortality. Eleven out of the sixty leaf litter bags containing amphipods had one mortality, and two leaf litter bags had two mortalities. All bags with amphipod mortality were replaced with new amphipod individuals.

Leaf litter decomposition can take several months in freshwater streams (Webster and Benfield, 1986), however, high levels of salt concentrations as a result of road deicer application during the winter months often spike concentrations and then gradually return to lower, more stable levels (Haq et al., 2018). Therefore, twenty-eight days, or approximately one month, was selected as the duration for this experiment in order to mimic conditions similar to those following road deicer runoff events in winter months and not the entire duration of leaf litter breakdown in freshwater ecosystems.

At the conclusion of the experiment, each leaf litter bag was removed from the microcosms and checked for amphipod mortality. Once mortality was noted, all amphipods were removed from the bags. Leaf litter disks remaining were also removed from the bags, sorted by species, and placed into separate, pre-weighed tins. The leaf disks were oven-dried at 70 degrees C and then re-weighed. Any amphipod-presence assigned leaf bags that had amphipod mortality at the end of the experiment were eliminated from the final results.

Figure 1. Experimental design includes 10 microcosms total, 5 inoculated with salt and 5 without salt. Each microcosm contained 12 mesh bags. Each mesh bag contained one of three leaf litter treatments and either the presence or absence of an invertebrate. The last two rows are only shown for the first column; however, they should be repeated for each column.



Statistical Methods

Leaf litter breakdown was calculated using a simple mass loss formula, in grams, for each leaf litter bag.

Mass loss = M_f - M_i

Where M_i is the initial mass (g) and M_f is the final mass (g). Two approaches were taken to analyze patterns in mass loss among the various treatments.

First, the normality of the data was tested by checking the assumptions of parametric statistical tests. Once the assumptions were confirmed, a three-way ANOVA was performed to examine the interactive effects of the three treatments (chloride treatment, leaf litter treatment, and invertebrate treatment). However, no statistically significant interactive effects were found at the third level (chloride treatment x leaf litter treatment x invertebrate treatment). Then, a two-way ANOVA was performed to test the interactive effects between each treatment combination (chloride treatment x leaf litter treatment, leaf litter treatment x invertebrate treatment, chloride treatment x invertebrate treatment, etc). The second statistical analysis tested for a diversity effect from the mixed leaf litter treatment. A one-way t test was used to compare the mean of the single-species leaf litter treatments to the mean of the mixed-species leaf litter treatments. The parametric statistical tests and ANOVA testing was carried out in R while the one-way t-tests were conducted using Microsoft Excel.

RESULTS

Mass loss rates varied between different treatments, but one strong trend remained consistent across all treatments. Regardless of invertebrate or chloride presence, the single-species maple litter lost more mass with an average of approximately 49% mass loss (Table 1). In comparison, the single-species beech litter lost, at a maximum, approximately 23% with the presence of invertebrates (Table 1). Maple leaf litter exhibited increased decomposition rates compared to beech leaf litter in both single-species and mixed leaf litter treatments. Maple litter decomposition made up 79-83% of the total litter decomposition in mixed litter treatments (Table 1). Overall, there was a statistically significant difference in mass loss between the leaf litter treatments (Table 2).

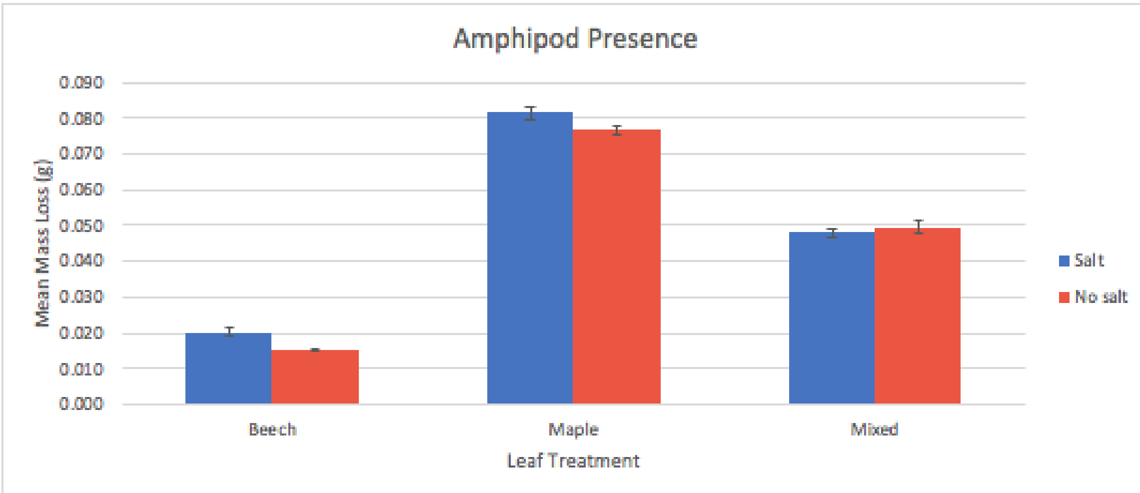
The presence of *Gammarus sp.* increased mass loss among all treatments regardless of leaf litter type or chloride presence. This result was most pronounced in the single-species maple litter treatment with no salt where the presence of the amphipod shredder resulted in an approximately 11% increase in mass loss compared to the equivalent no amphipod treatment.

Table 1. Mass loss results in percentages for all treatments. The percent maple to beech represents the proportion of the total mass loss for the mixed leaf litter treatment that each individual litter species comprised by mass.

			Percent Mass Loss	Percent Maple / Percent Beech
Salt	Amphipod	Beech	22.99%	
		Maple	51.63%	
		Mixed	40.28%	83% / 17%
	No Amphipod	Beech	20.05%	
		Maple	46.30%	
		Mixed	32.60%	79% / 21%
No salt	Amphipod	Beech	18.12%	
		Maple	53.89%	
		Mixed	41.46%	81% / 19%
	No Amphipod	Beech	14.22%	
		Maple	42.49%	
		Mixed	34.46%	80% / 20%

The presence of chloride altered feeding rates differently than predicted. The hypothesis stated that feeding rates of invertebrates would decline under the presence of increased chloride concentrations. However, the opposite effect was observed in single-species litter treatments. For both single-species maple and beech litter treatments, mass loss increased under the presence of chloride (Figure 2; Figure 3). Conversely, in the mixed litter treatments, mass loss decreased under the presence of chloride which supported our hypothesis (Figure 2; Figure 3). These results were consistent in both amphipod presence and absence treatments (Figure 2; Figure 3). Thus, regardless of invertebrate presence, chloride only decreased mass loss in heterogeneous leaf litter assemblages.

Figure 2. a) Mean mass loss (g) for each amphipod present leaf litter treatment with and without the presence of salt. b) Mean mass loss (g) for each amphipod absent leaf litter treatment with and without the presence of salt. Bars represent mass loss (g) +/- 1 standard error.



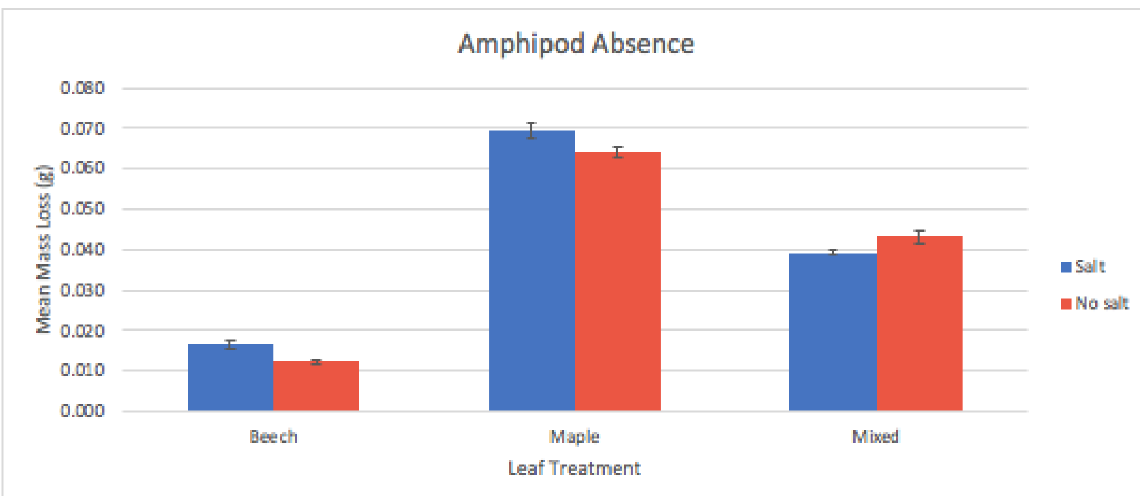


Figure 3. Mean mass loss (g) organized by treatment type. Bars represent mass loss (g) +/- 1 standard error. For the mixed litter treatment, the amount of mass loss for each individual species is shown by separate colors.

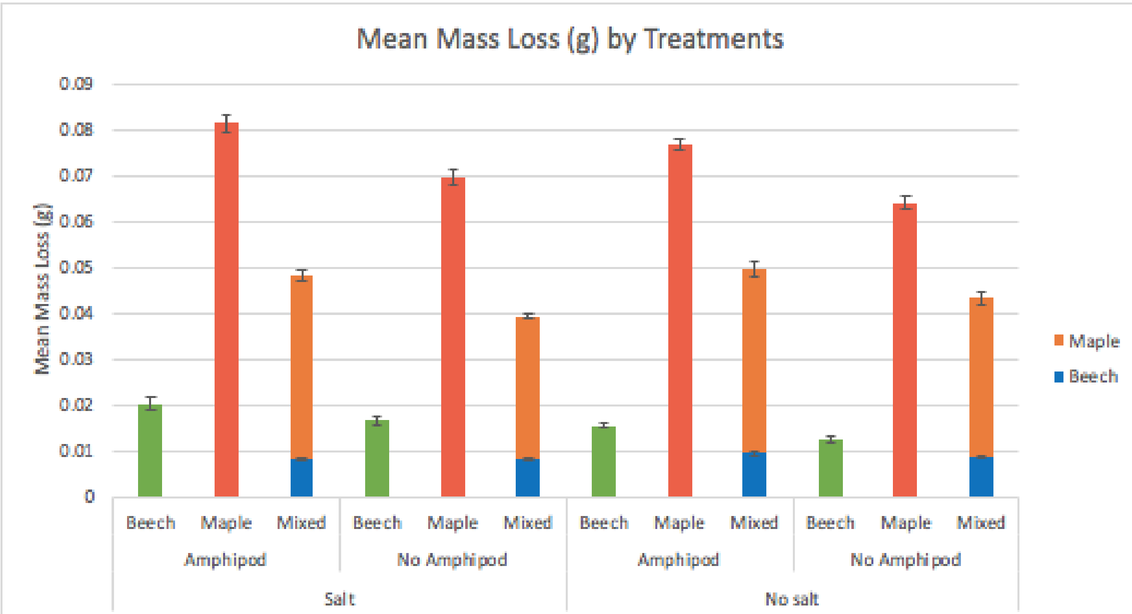


Table 2. Two-way ANOVA results for the interactive effects of the leaf litter treatments (single-species maples, single-species beech, mixed species; "Leaf Type"), chloride treatments (none, present; "Salt"), and invertebrate treatments (presence, absence; "Amphipod").

	Source	df (numerator/ denominator)	*F*	*P*
Mean Mass Loss (g)	Leaf Type	2	1984.73	2.00E-16
	Salt	1	6.10	0.0153
	Amphipod	1	113.32	2.00E-16
	Leaf Type x Salt	2/1	11.95	2.38E-05
	Leaf Type x Amphipod	2/1	12.47	1.57E-05
	Salt x Amphipod	1/1	0.12	0.6619

A three-way ANOVA revealed no significant interactions between all three treatments. However, the results of the two-way ANOVA revealed a significant difference between the interactive effects of the leaf litter treatments and the amphipod treatments (Table 2). To better understand invertebrate feeding between the single-species leaf litter treatment and mixed species treatments, one-way t-tests were performed to understand the role of preferential feeding. The results of the tests showed that in the amphipod absent treatments, regardless of chloride presence, there was a statistically significant difference indicative of a diversity effect (Table 3; Gessner et al., 2010). Conversely, in the presence of an amphipod, the results were not statistically significant and no diversity effect was observed (Table 3). Thus, the presence of an invertebrate eliminated the diversity effect regardless of chloride concentration.

Table 3. Results of One-Way T-test for each treatment combination. Statistically significant results indicate a diversity effect.

		P	< 0.5
Salt	Amphipod	0.074	N
	No Amphipod	0.041	Y
No Salt	Amphipod	0.055	N
	No Amphipod	0.007	Y

DISCUSSION

Increased urbanization introduces new pollutant sources and increases stress in freshwater ecosystems. One growing pollutant of concern is road deicer which is expressed as increased chloride concentrations in the water column (Kaushal et al., 2005). As impervious surface cover increases mean chloride concentration has been shown to also increase to levels at or above tolerance for many freshwater species

(Kaushal et al., 2005; Tiwari & Rachlin, 2018). Benthic invertebrates are subject to the stressors that enter their freshwater environments including increased chloride levels. Recent studies in the mid-Atlantic region showed that high levels of chloride stress in the ecosystem negatively affected invertebrate communities (Morgan et al., 2012; Stranko et al., 2013). Chloride tolerance varies greatly both within and across freshwater species. Freshwater fish have shown negative response to increased concentrations as low as 33 mg/L while most invertebrate species can tolerate concentrations well above 240 mg/L (Blasius & Merritt, 2002; Morgan et al., 2012).

For this experiment, chloride concentrations were measured and maintained around 600 mg/L. At this level, chloride stress can affect their internal processes, alter feeding rates, and reduce overall detritus breakdown rates (Blasius & Merritt, 2002; Swan & DePalma, 2012). Despite the findings in previous studies, elevated chloride concentrations did not decrease detritus processing rates for all treatments in this experiment. In this study, invertebrate feeding rates decreased under the presence of increased chloride only in the mixed-leaf litter treatments (Figure 3). Feeding rates, expressed as mass loss, increased under the presence of chloride in both the single-species maple and beech leaf litter treatments. This trend in increased mass loss under the presence of higher chloride concentrations was also observed in the amphipod absent treatments for the single-species leaf litter (Figure 3). Therefore, increased mass loss under the presence of increased chloride concentrations seems to have occurred independent from any invertebrate interactions. One potential explanation for this result is that increased chloride concentrations altered the water chemistry in a way that increased the overall breakdown of the primary and secondary compounds within the leaf litter itself. Some research found similar trends where increasing salinity can increase leaf litter decomposition to a certain extent (Connolly et al., 2014; Karavin et al., 2016; Zhai et al., 2021). Additionally, studies have found freshwater bacteria can have high salt tolerance and are not affected by increased salinity levels (Berga et al., 2017; Zhang et al., 2014). Perhaps low chloride concentrations combined with stable or even compensatory microbial activity contributed to the results. Overall, increased chloride concentrations did not have significant impacts on leaf litter decomposition in this experiment. Future research should consider performing this study with several concentrations of chloride in order to understand the gradient effect of increasing salinity on leaf litter breakdown without the presence of an invertebrate so one can better understand the role of invertebrate feeding on leaf litter decomposition under the presence of increased chloride conditions.

Compensatory feeding by invertebrates under limited resource diversity and preferential feeding under diverse litter conditions has been documented and can contribute to diversity effects in freshwater ecosystems (Swan & Palmer, 2006b; Gessner et al., 2010). In this study, invertebrates displayed both compensatory and preferential feeding strategies. Preferential feeding was expressed in mixed leaf litter treatments through increased mass loss of maple litter compared to the beech litter. When given the option, invertebrates preferentially chose to feed on maple leaf litter. According to Gessner et al (2010), compensatory feeding is defined as the increased consumption of poor-quality resources to ensure satisfaction of basic metabolic needs. By this definition, the results also suggest compensatory feeding strategies due to the limited diversity of leaf litter in the mixed litter treatments. Thus, a combination of compensatory and preferential feeding strategies were displayed but additional research is needed to examine the individual roles of compensatory and preferential feeding strategies.

The elimination of the diversity effect under the presence of the amphipod species suggests a mediation effect. The invertebrate served as the “mediator” variable in that it altered the interactive effects of the independent variable - leaf litter type - and the dependent variable- mass loss - (Agler & De Boeck, 2017). Shredder presence explains some of the variation in feeding rates between the single and mixed leaf litter treatments. The results of this study were complex and revealed the relationship between invertebrates and chloride presence was largely unexpected, but the relationship between invertebrates and the various leaf litter treatments was strong.

The two main objectives of this study were 1) to understand the influence of chloride on leaf litter decomposition and the role of invertebrates under those conditions and 2) to understand the influence of heterogeneous leaf litter on invertebrate feeding and decomposition under the presence of chloride. Though strong trends were observed between some treatments, the results were convoluted and suggest additional factors may have contributed to the results. Additionally, the influence of chloride on invertebrate feeding rates was much less than predicted based on similar research (Blasius & Merritt, 2002; Swan & DePalma, 2012). Despite the unexpected findings, this study contributes to the diversity effect literature and supports the notion that decomposers can mediate diversity effects in freshwater ecosystems. Future research should consider incorporating additional leaf litter species to create more diverse leaf litter assemblages than the ones used in this design. The next step in this research should also include additional chloride treatments of varying concentrations to better understand the interaction between the increasing salinity gradient and invertebrate feeding. As urbanization continues at a rapid pace, understanding the effects of stressors on freshwater environments will continue to be vital to community ecology.

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