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Effects of Road Salt on Photosynthetic and Enzyme Activity of Stream Biofilms

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EFFECTS OF ROAD SALT ON PHOTOSYNTHETIC AND ENZYME ACTIVITY OF STREAM BIOFILMS

BY

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DEDICATION

To the three pillars in my life: God, my husband, and my children.

"For I know the plans I have for you," declares the LORD,

"plans to prosper you and not to harm you, plans to

give you hope and a future" Jeremiah 29:11.

DeWayne, you are an amazing, supportive husband

who continues to push me to do my best in everything.

I love you and thank you so much for always being there.

Charlotte and Lukas, you are the legacy of all my

hard work. "Mommy's homework" is done.

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ABSTRACT

Road salt application and salt-laden run-off are common, yet little information exists about its influence on periphyton. I measured extracelluar enzyme activity and photosynthetic activity of natural winter stream periphyton communities under various short-term laboratory salt exposures. The results of this study suggest that short-term exposure to increased concentrations of salt did not affect extracelluar enzyme activity but could temporarily reduce the photosynthetic activity of the periphyton. Further work will be necessary to understand the long-term effects of increased salt concentrations on periphyton and extracellular enzyme activity.

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INTRODUCTION

Winter roads are often maintained by the application of some form of chemical deicer, often road salt. Removing snow and salting of roads during wintry weather is used to increase the safety of commuters. Salt lowers the freezing point of water, thus preventing ice formation and encouraging melting (Oškinis & Kasperovičius, 2005). Maintenance of winter roads by applying salt was first introduced in the 1930s and has been used more frequently since the 1960s (Paschka et al., 1999). Several salts are used on roads. The most common are calcium chloride and sodium chloride (Environment Canada, 2001; Ramakrishna & Viraraghavan, 2005). Additives may be used to increase the effectiveness of the salt (Novotny et al., 2008). In Washtenaw County, Michigan, the typical annual use of salt by the Washtenaw County Road Commission is 10,000 to 25,000 tons of salt applied on its ~ 2,000 miles of roadways (http://www.wcroads.org/services/operations/saltusage.htm, last accessed 04-30-2010). For example, in the 2006/2007 winter season, the Washtenaw County Road Commission used 16,837 tons of salt. These figures do not include road salt applications by the numerous municipalities within Washtenaw County, nor do they include applications by private individuals on sidewalks, driveways, and parking lots.

The clearing of snow from roadways, sidewalks, and driveways contributes to the salt entering nearby habitats (Environment Canada, 2001). Furthermore, aquatic habitats near roadways or other impervious surfaces acquire runoff containing a greater concentration of deicing chemicals (Novotny et al., 2008; Paschka et al., 1999; Environment Canada, 2001). High salt concentrations enter into streams and lakes through snowmelt runoff (Oškinis & Kasperovičius, 2005). This runoff can be either

direct (surface flow into a nearby surface water) or indirect (runoff that is detained or infiltrated into the aquifer then into nearby water systems). Increased conductivity in streams during winter months resulting from salt applications is common (Oškinis & Kasperovičius, 2005). Furthermore, some aquatic habitats do not have the ability to dilute the salts, which can influence the water chemistry of the system (Environment Canada, 2001). Lakes can experience a seasonal cycle of chloride related to road salt applications (Novotny et al., 2008), and salt runoff can alter water densities in lakes, leading to salt-induced lake stratification (Ramakrishna & Viraraghavan, 2005; Dodds & Whiles, 2010). Lakes in Washtenaw County have experienced similar impacts (Judd et al., 2005), and winter stream conductivities can be greatly elevated (Fig. 1).

Adding salt to an environment can influence the environment and its aquatic organisms. Increased salt within the environment can change the density of water and reduce the solubility of other molecules. Increased water density influences lake stratification (Judd et al., 2005; Novotny et al., 2008). In addition to impacting aquatic habitats, increased salt in the environment can influence ions found in the soil (Environment Canada, 2001).

Organisms that live in or near aquatic systems vary in their tolerances to salt. A wide variety of organisms have been studied in relation to their exposure to salt in the field and in the laboratory. These organisms include fish, zooplankton, and benthic invertebrates where laboratory studies investigated chloride toxicity in short term exposures (Environment Canada, 2001). Further studies using amphibians and road salt suggest that aquatic organisms react negatively to increased salt concentrations. Sanzo and Hecnar (2006) observed the influence of sodium chloride on larval wood frogs (*Rana*

sylvatica). They found that salt concentrations lowered survivorship and reproductive fitness. The frogs also displayed more physical abnormalities with increased concentrations of salt. In addition, Denoël et al. (2010) concluded that tadpoles (*Rana temporaria*) exposed to high salt concentrations had a slower movement speed than control tadpoles.

Macroinvertebrates also have been used to study the effects of salts on aquatic systems. Researchers have classified macroinvertebrates as tolerant or non-tolerant to salt (Williams et al., 1999). Blasius and Merritt (2002) found that macroinvertebrate community composition and mortality were affected by short-term exposure to elevated salt concentrations; some macroinvertebrates were more tolerant than others of higher concentrations of salt.

Diatom assemblages can indicate changes in conductivity of aquatic habitats. Indices have been created to reflect the conductivity ranges of ecosystems using diatoms (Blinn, 1993; Clavero et al., 2000). Increasing conductivity in established mesocosms decreased species richness and diversity in diatom assemblages. Decreased diatom diversity and species richness in relation to increased conductivity were also observed by Blinn (1993), who collected diatoms from 63 North American lakes. In addition, the researcher found a significant relationship between particular anions and diatom species in low and high conductivity environments. The presence of salt may hinder the growth of organisms or change the community composition within ecosystems (Herbst & Blinn, 1998). Growth of diatoms is generally reduced in the presence of higher salinity (Saro & Fritz, 2000). When excess salinity is introduced to an environment, organisms often need to allocate energy for self-maintenance rather than growth (Calow, 1991). Photosystem II

in algae can be inhibited by the presence of excess salt (Allakhverdiev et al. 2000; Lu & Zhang, 2000; Sudhir et al., 2005).

In addition, algae exposed to salt can exhibit decreased photosystem II activity and decreased oxygen production (Allakhverdiev et al., 2000; Lu & Zhang, 2000; Sudhir et al., 2005). The cyanobacterium *Synechococcus* sp. provides an example of how a photosynthetic aquatic organism adapts to ionic and osmotic stressors. Ionic and osmotic stressors have been studied to determine their influence upon Photosystem I (PSI) and Photosystem II (PSII) in this organism (Allakhverdiev et al., 2000). Cyanobacteria were exposed to 0.5M NaCl, and oxygen production due to PSII was measured. A rapid decrease of oxygen production was observed after 0.5, 1, or 5 hours exposure to 0.5M NaCl. Following the shortest exposure, washing cells and returing them to a normal culture medium resulted in complete recovery of photosynthetic activity. After the 1-hour NaCl incubation, the cells recovered partially when returned to normal culture medium. After the 5-hour NaCl incubation, the cells showed no recovery when washed and returined to normal culture medium. Similar experiments using exposure of cells to non-ionic sorbital indicated that both ionic and osmotic effects can contribute to reduced oxygen production caused by exposure to salts such as NaCl (Allakhverdiev et al., 2000).

Changing salinity is a form of osmotic stress that changes the osmotic equilibrium (Dodds & Whiles, 2010). Organisms exposed to higher salinity levels also experience osmotic water loss. If equilibrium cannot be established, the pressure from the osmotic gradient will dehydrate the cells and they will collapse (Dodds & Whiles, 2010). In addition, the increased ion concentrations can influence electron transport activities and thylakoid membrane protein function. The inhibition of protein function suggests the

potential for inhibition of PSII activity (Sudhir et al., 2005). Furthermore, an increase in salinity may cause metals to be more toxic by influencing rates of metal adsorption and uptake (Borchardt, 1996)

The rate and duration of salt inputs to aquatic environments may be moderated by soils. Negatively charged chloride ions rapidly pass through the negatively-charged soil (Environment Canada, 2001). This will contribute to increased salinity in the soil. The positive sodium ions will also move through the soil, but at a slower rate, and can displace calcium and magnesium cations. Long-term salt application may result in soils with elevated salinity; such soils will leach salt into nearby pools or streams, causing elevated aquatic salt concentrations at various times of the year (Ramakrishna & Viraraghavan, 2005; Kelly et al., 2008).

Periphyton is the community of microorganisms (e.g., algae, bacteria) that grows attached to surfaces in aquatic environments. Benthic algae are an important component of periphyton in streams, contributing to the base of the food web and serving as water quality indicators (Hauer & Lamberti, 1996). Benthic algae are important primary producers in streams and serve as a food source for stream invertebrates (Borchardt, 1996). Furthermore, they are necessary as chemical modulators, stabilizers of substrata, and providers of habitats for other organisms (Borchardt, 1996). There are several factors that could limit benthic algae within a stream including "light, temperature, current, substrate, scouring by floods, water chemistry and grazing" (Allan & Castillo, 2007).

Periphyton help transfer organic matter through the aquatic habitat by utilizing extracellular enzymes to hydrolyze organic molecules into smaller ones for microbial use (Pohlon et al., 2010; Allan & Castillo, 2007). Both bacterial and algal components of

periphyton communities produce and secrete extracellular enzymes. Enzyme activity can indicate a shift in physiological state of algae and bacteria. Ratios of the activities of N-, P- and C-acquiring extracelluar exzymes can be used to infer whether microrganisms are N-, P-, or C-limited (Rier et al., 2011; Sinsabaugh et al., 2010). The release of such enzymes can also influence dissolved organic carbon concentrations within a stream (Allan & Castillo, 2007), and enzyme activity is related to decomposition rates of detritus (Sinsabaugh et al., 1994; Sinsabaugh & Linkins, 1993).

Little is known about the influence of salinity arising from road salt application on the functioning of stream periphyton. Studies have shown that high salinities have an effect on the overall functioning of stream biota within a stream (Silva et al., 2000). Photosynthesis of freshwater algae can be enhanced by moderate ion concentrations (<~5800 μS cm^{-1}) and reduced by higher levels (~ 10,200 μS cm^{-1}; Silva & Davies, 1999). Enzyme activity could be affected by chemical conditions within the matrix of the biofilm (Rier et al., 2007). However, very little research has focused on the influence of conductivity on enzyme activity. In natural systems, elevation of ionic strength via addition of humic acids inhibited extracellular enzyme activity (Dudley & Churchill, 1995).

The purpose of this study is to determine if periphyton photosynthetic activity and extracellular enzyme activity could be influenced by road salt run-off. Thus, the hypothesis was that a reduction of periphytic photosynthetic and enzymatic activity would occur with increased conductivity. This was tested by conducting laboratory experiments that exposed natural winter periphyton communities to realistic salt concentrations.

MATERIALS AND METHODS

Study Site

The study was conducted in Paint Creek (N42.21502, W83.63494), just downstream of the Paint Creek wetland retention basin within the Stony Creek (Michigan, USA) watershed. Water temperature, specific conductance (YSI model 63), depth, and photosynthetically-active radiation (LI-Cor model 189) above the water surface were recorded during sampling visits. Stream water was also collected for experimental use and measurements of total phosphorus (TP), ammonia (NH_4^+), nitrate and nitrite (NO_3^-+NO_2^-) were made. Water samples for nutrient assays were frozen until analysis (method of Lind, 1985) and samples for NH_4^+, and NO_3^-+NO_2^- analyses were filtered (0.7-µm pore size glass-fiber filters) prior to freezing.

Experiment One (February 6, 2007)

Glass fiber filters were prepared by sandwiching the filters between two plexiglass plates, one of which contained openings through which periphyton colonized the filters (see Kahn & Wetzel, 1999; Francoeur & Wetzel, 2003). The plexiglass plates were wired to bricks and placed on the streambed. After four weeks of periphyton growth (February 6, 2007), the glass fiber filters were retrieved and returned to the lab. Care was taken to avoid losses of periphyton during collection and transport.

In the lab, four sections were cut from the filters using a cork borer (#10, 1.5 cm diameter). These samples were preserved in 5% gluteraldehyde and stored at 4°C in darkness for later microscopy to determine algal community composition. Preliminary scans indicated that all algae present were diatoms, so samples were cleaned and mounted

for microscopy (method of Carr et al., 1986). A minimum of 100 diatoms per sample were identified and enumerated (1000×).

Three replicate sections (each ~176.7mm^2) of glass fiber filters were collected and frozen for subsequent chlorophyll analysis by method of Biggs and Kilroy (2000). Chlorophyll was extracted in 90% ethanol (80°C for 5 minutes), followed by overnight (4 °C, darkness) steeping. Samples were then lightly centrifuged to ensure settling of particulate matter, and chlorophyll content was quantified from spectrophotometric absorbance at 665nm and 750nm, with acidification to correct for pheopigments.

The collected stream water was used to generate two salt treatments: ambient (1637.2 µS cm^{-1}, unamended site water) and 35,320 µS cm^{-1} (site water amended with reagent-grade NaCl). Seven replicate sections (~176.7 mm^2) of glass fiber filter substrata were subjected to each salt treatment. The substrata were immersed in 5.0 mL of the treatment solution for 30 minutes before the first measurement (0 hours) of dark-adapted photosystem II yield (Walz Diving PAM, see Consalvey et al., 2005). Additional dark-adapted yield measurements were collected at 3 hours, 6 hours, and 24 hours. For the entire course of the experiment, all samples were held in darkness at 5 °C.

Experiment Two (March 6, 2007)

Naturally-senesced *Typha* litter was collected from the Paint Creek wetland < 100 m upstream of the sampling site and placed in floating mesh trays (see Francoeur et al., 2006). After eight weeks of periphyton growth (March 6, 2007), the *Typha* trays were retrieved and returned to the lab. Care was taken to avoid losses of periphyton during transport.

Algal biomass (as chlorophyll a, n = 3) and community composition (n = 4) were quantified as in Experiment 1, with the exception that known lengths of *Typha* litter were cut for quantitative analyses (see Francoeur et al., 2006). An additional four litter sections were frozen for subsequent analysis of fungal biomass (method of Francoeur et al., 2006; Table 2). Dry mass of the litter and associated microbial communities was measured by drying litter sections (105 °C for 24 h) in pre-weighed, pre-ashed aluminum pans, cooling in a dessicator, and weighing. Ash-free dry mass was measured by ashing (500 °C for 2 h) the dried litter, cooling in a dessicator, re-weighing, and subtracting the ash weight (n=10).

Activities of three microbial extracellular enzymes (leucine aminopeptidase, β-glucosidase, and phosphatase) were compared at two levels of salinity: 2009.2 μS cm^{-1} (ambient stream water) and ~60,000 μS cm^{-1}. Six replicates of the *Typha* litter sections and two litter-free blanks were used for each combination of salt treatment and enzyme type. Replicate litter sections (1.7 cm long) were pre-incubated in ambient or 33,040 μS cm^{-1} stream water for 1 h (5°C, in darkness) before enzyme activity assays. The assays followed the method and conditions of Francoeur et al. (2006), which are saturating for these microbial communities. The containers containing the litter sections were aspirated of any liquid and 2 mL of 1000 uM stock solution, and one milliliter of 179,300 μS cm^{-1} or 2009.2 μS cm^{-1} stream water were added to each container. The final concentration of extracellular enzyme substrate was 666 uM stock solution, and final salt concentrations were approximately 60,000 μS cm^{-1} (elevated salt treatment) or 2009.2 μS cm^{-1} (ambient stream water treatment). Samples were incubated for 30 minutes in the dark at 5°C.

Aliquots from each vial were added to a 96-well plate with equal volumes of pH 10 buffer and measured fluorometrically .

Experiment Three (March 13, 2007)

On March 13, 2007, natural rock substrata were collected from the Paint Creek study site and returned to the lab with care to avoid losses of periphyton during transport. In the lab, replicate sections (~100 mm^2) of intact periphyton mat were removed from the rock surfaces. Algal biomass (as chlorophyll a, n = 3) and community composition (n = 4) were quantified as in Experiment One. An additional 3 sections were preserved in 4% formalin and stored at 4°C in darkness for subsequent analysis of bacterial abundance. Bacterial samples were disrupted by probe sonication (Francoeur et al., 2006), stained with SYBR Gold, mounted on 0.2 μm Anodisc filters in anti-fade reagent (see Noble & Fuhrman, 1998), and examined using epifluorescence microscopy (1000×). At least 350 cells and 10 fields were examined for each sample.

Three salinity treatments were made by adding NaCl to stream water: 1609.8 μS cm^{-1} (unamended stream water), 4428 μS cm^{-1}, and 32750 μS cm^{-1}. Seven replicate periphyton sections were immersed in 5.0 mL of each of the desired salt treatments. Incubation and photosystem II yield measurements were conducted as described above, with the exception that after 6 hours of incubation, all sections were removed from incubation water, rinsed with unamended stream water, and placed into unamended stream water. Incubation resumed and photosystem II yield measurements were made at 0.5 hours after rinsing, 3 hours after rinsing, and 21 hours from rinsing.

Statistical Analysis

Experiment One. A Repeated-Measures ANOVA (RMANOVA) was conducted to test the hypothesis that potential algal photosynthetic activity (dark-adapted photosystem II yield) differed between ambient and elevated salt treatments over the 24 hour experiment. Additionally, two-sample separate-variance t-tests were used to test for differences between ambient and elevated salt treatments at each individual measurement period.

Experiment Two. Two-sample t-tests were used to test the hypothesis that activities of individual extracellular enzymes (leucine aminopeptidase, β-glucosidase, and phosphatase) differed between ambient and elevated salt treatments.

Experiment Three. RMANOVA was used to test for differences in dark-adapted photosystem II yield between salt treatments over the entire experiment. Bonferroni-based comparisons were used to identify differences between specific elevated salt treatments and the control. One-way ANOVAs with Tukey HSD multiple comparisons were used to test for differences among salt treatments at each individual measurement period. In addition, subsequent RMANOVAs considered only the pre-rinse measurements (0-6 h, when algae were exposed to three distinct salt treatments), and only the post-rinse measurements (6.5- 21.5 h, when all algae were in ambient salinity water). Finally, paired t-tests were used to test the hypothesis that dark-adapted photosystem II yield within individual salt treatments differed between the 6 h and 6.5 h measurements (i.e., immediately pre- and post-rinse).

Data were transformed as necessary to reduce heteroscedasticity. All statistical analyses were conducted using SYSTAT, Version 10 (Systat Software, Point Richmond, California).

RESULTS

Environmental Conditions

Over the course of the in situ incubations (January 4, 2007; January 18, 2007; January 29, 2007; February 2, 2007; February 22, 2007; March 6, 2007; March 13, 2007) the mean nutrient levels (± 1 SE) in Paint Creek were 2.29± 0.56 Nitrate-Nitrite mg/L (n=6); 0.15673 ± 0 NH_4^+ mg/L (n=6); 58.3 ± 29.3 TP µg /L (n=6). Average stream water temperature was reported, and mean specific conductance was collected in situ or in the lab (Table 1). Algal biomass was greatest in the Experiment Three communities, moderate in the Experiment One communities, and lowest in the communities used in Experiment Two (Table 2). The only algae found were Bacilliarophyta. There were limited algal communities found in Experiment Two. The ash free dry mass mean (± SE) found within a sample taken on March 6 (Experiment Three) was 0.05 (± 0.013) mg cm^{-2}, and the dry mass mean (± SE) was 0.03 (± 0.01) mg cm^{-2}.

Photosynthesis Experiments

In Experiment One, exposure to salt amended stream water (35,320 µS cm^{-1}) significantly reduced photosynthetic yield of photosystem II (Fv/Fm) over the entire 24-hour experimental period ($p<0.001$, Figure 2) and at each individual sampling time (p always < 0.001).

In regard to the full photosynthetic yield data set of Experiment Three, repeated-measures ANOVA detected significant differences among the three salinity levels ($p<0.001$) and a strong time x salinity interaction ($p < 0.001$) (Figure 3). Both the medium (4,428 µS cm^{-1}, $p < 0.004$) and high (32,750 µS cm^{-1}, $p < 0.001$) treatments differ from the ambient-salinity control; however, the time x salinity interaction was only

apparent when comparing the high salinity and ambient treatments ($p < 0.001$); the ambient and medium salinity treatments displayed the same pattern over time ($p = 0.201$). Potential photosynthetic yield differed significantly among salinity treatments in every pre-rinse (i.e., times 0, 3, and 6) sampling period (one-way ANOVA p always < 0.001).

A repeated-measures ANOVA considering only the pre-rinse sampling periods (i.e., times 0, 3, and 6) detected significant differences among salinity treatments ($p < 0.001$); both high ($p < 0.001$) and medium ($p = 0.006$) salinity treatments differed from the ambient treatment. The high salinity treatment was significantly different from the ambient (p always < 0.001) and medium salinity treatments (p always < 0.001); however, the ambient and medium salinity treatments did not differ at individual pre-rinse sampling periods (p always > 0.424).

Comparison of immediately pre-rinse (6 h) and immediately post-rinse (6.5 h) measurements showed that photosystem II yield in the ambient water (1609.8 $\mu S\ cm^{-1}$) and low salinity (4,428 $\mu S\ cm^{-1}$) treatments increased slightly, but the differences were not significant ($p=0.071$ and $p=0.797$, respectively). However, the photosynthetic yield of the high salinity treatment (32,750 $\mu S\ cm^{-1}$) increased significantly during this timeframe ($p<0.001$, Figure 3)

A repeated-measures ANOVA considering only the post-rinse sampling period did not differ significantly among salinity treatments ($p = 0.060$). Potential photosynthetic yield in each of the post-rinse sampling periods (i.e., times 6.5, 9.5, and 21.5) reflected this similarity among salt treatments (one-way ANOVA p always < 0.086).

Extracellular Enzyme Activity Experiment

Extracellular enzyme activities (leucine aminopeptidase, phosphotase and beta-glucosidase) were not affected by high (~60,000 μS cm^{-1}) salinity ($p = 0.413$, $p=0.902$, $p=0.899$, respectively; Figure 4).

DISCUSSION

The purpose of this study was to determine the short-term effects of salt stress on lotic microbial function in order to evaluate possible ecological effects from road salt run-off. We approached this task by exposing natural periphyton communities to simulated salting events. Laboratory conditions were calibrated to local environmental data in order to provide realistic salt concentrations and temperatures.

Potential photosynthetic activity showed a rapid reduction of activity when exposed to high, but realistic, salt concentrations. When high salt exposure samples were rinsed with ambient salinity stream water, a rapid recovery of photosynthetic activity was observed. In a Canadian gravel-bed river, Silva and Davis (1999) reported stimulation of natural lotic periphytic productivity by slightly increased salinity (up to ~ 6,000 $\mu S\ cm^{-1}$), then strong reductions in periphytic productivity by salt stress in the highest salinity treatment (~ 10,500 $\mu S\ cm^{-1}$). In contrast to Silva and Davis (1999), I did not observe any stimulation of photosynthetic activity at medium levels of salinity (4,428 $\mu S\ cm^{-1}$); the data indicated that these medium salinity levels had either no effect or were slightly inhibitory. This could have been from the osmotic stress from exposure to the concentration of the salt. Despite this inconsistency between the studies, high salinity results from this study are broadly similar to those of Silva and Davis (1999), despite their high salinity treatment being well below my high salt concentration (~29,900 - 35,320 $\mu S\ cm^{-1}$).

Not only was a salt-induced rapid decline observed in potential photosynthetic ability, but also a rapid recovery when high concentrations of salt were rinsed and replaced with unamended stream water. This general pattern of rapid decline and

recovery appears superficially similar to the results of Lu and Zhang (2000), who observed instantaneous decline, then recovery of photosynthetic activity of the cyanobacterium *Spirulina plantensis* from salt exposure. However, the recovery from salt stress in their experiment was spontaneous, as it did not require removal of salt from the medium, perhaps the result of sodium being actively excluded from the cyanobacterial cells (Lu & Zhang, 2000), whereas in my experiment, recovery was due to removal of algae from the salt solution. Lu and Zhang (2000) also reported that after 4 hours, there was a second, light-mediated depression of photosystem II activity by salt. This phenomenon was something that would not have been able to be observed in my experiment, as the samples were incubated in darkness. However, spontaneous recovery of photosynthetic activity during my experiments was not observed; thus, there is no way that a secondary light-mediated inhibition could have occurred. It is likely that the large differences in algal community composition between our experiments (natural algal communities almost exclusively composed of diatoms) and the purely cyanobacterial cultures of Lu and Zhang (2000) caused this disparity in spontaneous photosystem II recovery. The fact that the cyanobacterial spontaneous photosystem II recovery observed by Lu and Zhang (2000) was later reversed by light exposure strongly suggests that this apparent spontaneous recovery was dependent on the use of alternative photosynthetic electron pathways unique to cyanobacteria (Lu & Zhang, 2000). Such pathways are lacking in plants and eukaryotic algae (see Campbell et al., 1998), and thus this phenomenon would not be expected to occur in algal communities dominated by eukaryotic diatoms.

A more critical finding of Lu and Zhang (2000) is the decoupling of photosystem II activity and photosynthetic oxygen evolution by salt exposure. Should this decoupling be a common phenomenon, then rapid measures of photosystem II activity would not be good proxies for traditional photosynthesis assays. However, the discrepancy observed by Lu and Zhang (2000) appears to entirely result from the apparent spontaneous recovery of photosystem II activity. As discussed above, this phenomenon is likely limited to cyanobacteria. Furthermore, this apparent spontaneous recovery can be easily distinguished by its sensitivity to light. Exposure to 100 μmol m^{-2} s^{-1} of light for several hours completely eliminated apparent spontaneous photosystem II recovery (Lu & Zhang, 2000). Thus, rapid, PAM-based measurement of photosystem II activity should generally provide an adequate estimate of eukaryotic algal photosynthetic performance under salt stress.

A similar decrease in photosynthetic oxygen production by *Synechococcus* sp. exposed to 0.5M NaCl was observed by Allakhverdiev et al. (2000). The cyanobacteria were rinsed after 0.5, 1 and 5 hours exposure to salt and the oxygen activity was measured. The samples that were rinsed after 0.5 hours of salt exposure were able to recover following a short exposure time to salt. The sample rinsed after 5 hours of salt exposure did not recover in their oxygen evolving activity of photosystem II. This lack of recovery is consistent with what Lu and Zhang (2000) found. In contrast, my study showed full recovery of photosyntheitc activity in a diatom-dominated periphyton community following a six hour exposure of 33,040 μS cm^{-1} salt. Taken together, these studies suggest that more work is required to understand the effects of salt on algae, as different species may react differently when exposed to various levels of salt.

In contrast to the strong responses observed for photosynthesis, extracellular enzyme activities showed no significant response to salt exposure. This could imply that there was no immediate effect of salt on decomposition. Enzymes are limited in what conditions they may function optimally within an environment and are limited by temperature, pH, concentration of ions, and salt (Audesirk et al., 2010; Tobin & Morel, 1997). Perhaps the biofilm nature of the extracellular enzymes buffered their activity against short-term salt exposure.

For determining the larger ecological implication of higher conductivity levels, a systems approach is necessary. Within Paint Creek, there were no rapid changes in the extracellular enzyme activity. However, potential photosynthetic activity was rapidly inhibited by high salt exposure but was also rapidly reversed by the removal of salt stress. This would suggest that if the salt exposure were temporary and short, there would be little to no long-term functional harm to stream microbes. Blasius & Merritt (2002) also support this finding using macroinvertebrates. Several species of macroinvertebrates were tolerant of short-term salt exposure. Furthermore, Silva & Davies (1999) also observed rapid recovery of macroinvertebrates following short-term exposure to salt. Rapid recovery of microbes following salt exposure was also observed in my study. However, there could be an effect on the production of other stream microbiotia (e.g., bacteria, fungi, or protists), but we did not measure these other ecosystem components.

This study was designed to measure short-term physiological responses to salt exposure. Long-term salt exposure would be predicted to change species composition within periphyton communities. If the community were changed to include more halotolerant species because of the long-term exposure to salt, we could then expect some

change in ecological performance. Because of the very different mechanisms causing short-term (rapid physiological responses of existing taxa) and long-term (community shifts to halotolerent taxa) responses to salt exposure, it is difficult to extrapolate the results to long-term whole-stream production and decomposition. Little is known about the influence of long-term exposure of salt upon streams and benthic community development.

Lack of short-term effects on lotic microbial community function does not imply that short-term salt inputs are harmless to all freshwater ecosystems. In contrast to stream communities, where pulses of salt-rich water would be an acute problem during snowmelt, salt exposure in lake systems is a chronic impairment and can change physical, chemical, and biological processes of a lake. Increased salt concentrations introduced from road salt can create a lake with conductivity stratifications, thus preventing turnover from occurring in the spring and fall (Novotny et al., 2008). High density salt water accrues in the hypolimnion layer of the lake (Judd et al., 2005). Prevention of turnover has detrimental effects on a lake's water quality (Novotny et al., 2008). The turnover process in a lake helps the lake keep the benthic regions oxygenated, prevents anoxic regions from developing, and promotes natural nutrient availability (Judd et al., 2005; Novotny et al., 2008) An increased anoxic region within a lake in the fall reduces the extent of suitable habitat for fish in the winter (Novotny et al., 2008). Furthermore, altered nutrient availability may affect the community composition of the lake (Judd et al., 2005). If salt concentrations in the sediments of a lake increase, heavy metals may be released from the sediment into the water (Novotny et al., 2008). As chloride concentrations have correlated with increased salt concentrations in the state of

Minnesota since the 1950s, there appears to be a trend of deceasing health of lakes due to the application of road salt (Novotny et al., 2008).

Large but realistic short-term salt exposures immediately caused reductions in potential photosynthesis of lotic periphyton. Extracellular enzyme activities were not affected, and photosynthetic impairment was rapidly and completely reversible by rinsing with ambient, low-salt streamwater. Thus, individual short-term salt exposures do not appear to cause permanent harm to lotic periphyton.

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Table 1. Environmental conditions at the Paint Creek field site. Values are means (± 1 SD) of six measurements made from 4 January – 13 March 2007). Asterisk indicates water samples were taken from stream and water temperature and conductance were measured upon returning to lab. n.d. = not determined.

	January 4, 2007	**January 18, 2007**	**January 29, 2007**	**February 2, 2007**	**February 6, 2007 (Experiment 1)**	**February 22, 2007**	**March 6, 2007 (Experiment 2)**	**March 13, 2007 (Experiment 3)**
Water Temperature (°C)	4.82 ± 0.04	3.7	0.65 ± 0.07	1.76 ± 0.37	3.6 ± 0.12	3.6 ± 0.12	5.68 ± 0.16**	12.46 ± 0.05**
Specific Conductance (µS/cm)	852.8 ± 1.9	1377.4 ± 20.0	n.d.	1805.8 ± 10.3	1637.2 ± 13.4	2256.6 ± 11.3	2009.2 ± 2.5**	1609.8 ± 3.2**
NO3+NO2 (mg/L)	2.67	2.99	2.30	1.49	n.d.	1.43	n.d.	1.83
NH4 (mg/L)	0.159	0.160	0.156	0.140	n.d.	0.165	n.d.	0.160
TP (µg/L)	105	65	25	30	n.d.	55	n.d.	70

Table 2. Mean (± 1 SE) algal biomass, fungal biomass, and bacterial abundance of periphyton communities used for the three experiments. n.d. = not determined.

	February 6, 2007	**March 6, 2007**	**March 13, 2007**
Algal Biomass (mg Chl a / m2) (n=3)	37 ± 6	16 ± 8	86 ± 12
Fungal Biomass (mg/g dwt.) (n = 4)	n.d.	53.15 ± 7.6	n.d.
Bacterial Abundance (x 10^{10} cells L^{-1}) (n = 3)	n.d.	n.d.	5.89 ± 4.47

Figure 1. Conductivity measurements (µS cm^{-1}, n = 1456, 1995-2006) from Huron River tributaries in Washtenaw County, MI and surrounding region. Data are sorted highest to lowest, and are courtesy of the Huron River Watershed Council (HRWC.org).

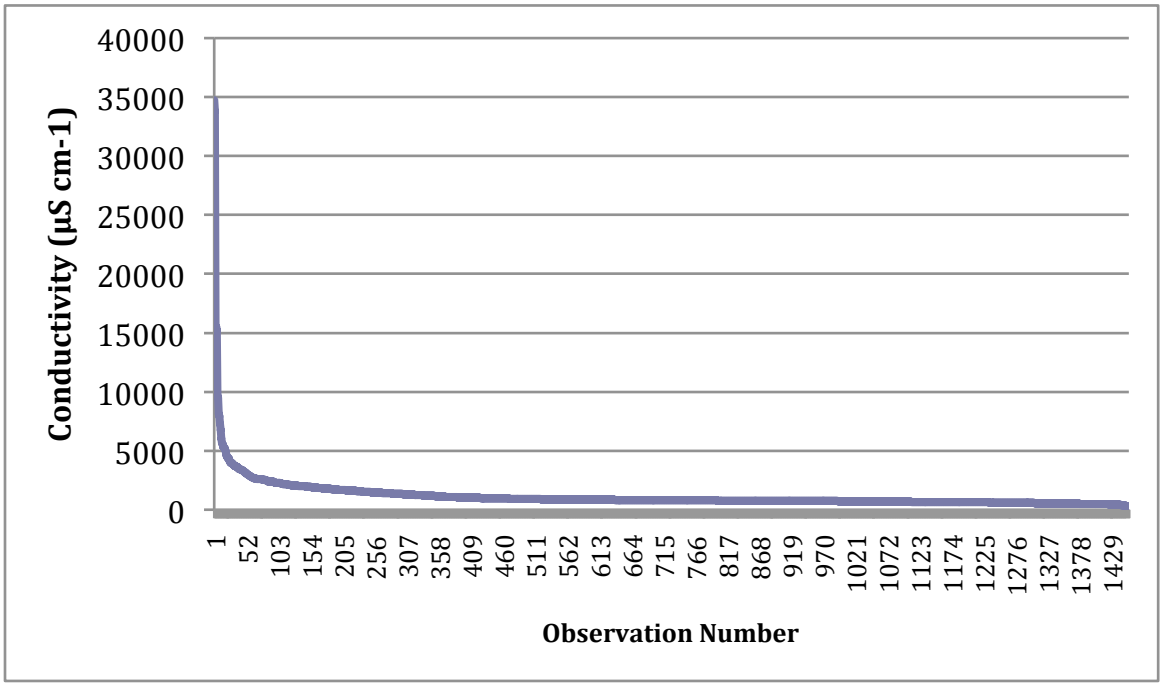

Figure 2. Effects of conductivity on dark-adapted photosystem II yield (Fv/Fm) in experiment one ($p<0.001$). Values are means ± 1 SE (n =7).

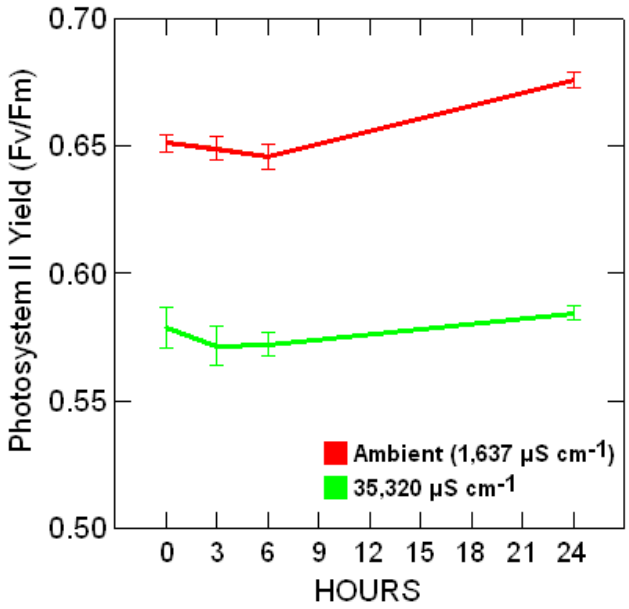

Figure 3. Effects of conductivity on dark-adapted photosystem II yield (Fv/Fm) in experiment three. Photosystem II yield measurements means were significantly different before a rinse ($p<0.001$), but not significantly different following the rinse at 6 hours and before 6.5 hours ($p=0.061$).

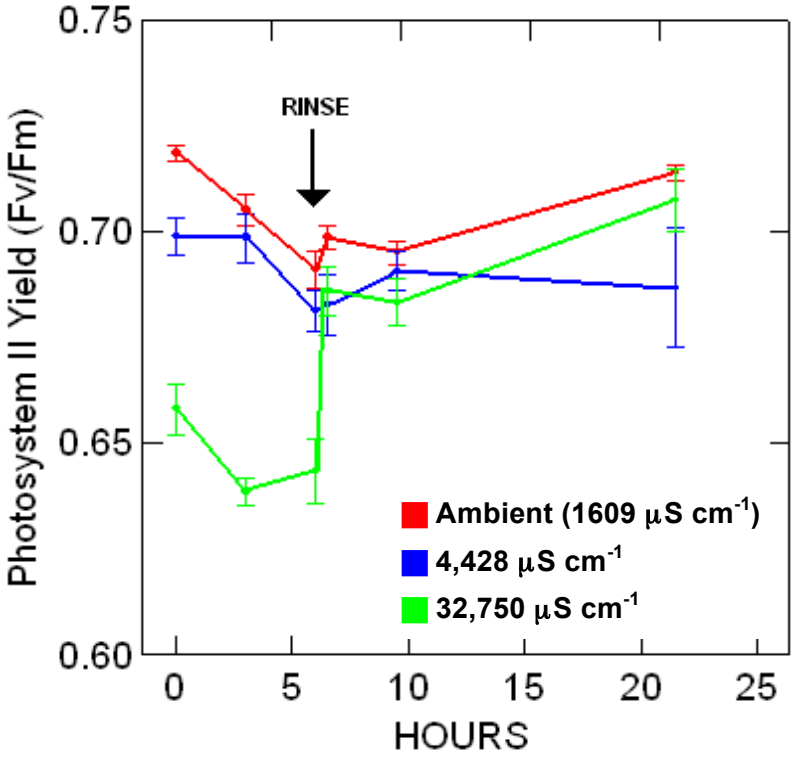

Figure 4. Mean extracellular enzyme activity (nmol cm $^{-2}$ h^{-1}) in conductivity treatments at ambient stream water (2009.2 µS cm^{-1}) and salt-amended stream water (~60,000 µS cm^{-1}).