**USING PAM FLUOROMETRY TO DETERMINE SALINITY STRESS IN A NATURAL PERIPHYTON COMMUNITY DURING A SHORT-TERM LABORATORY EXPOSURE**

by

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## ABSTRACT

Road salt application is a common practice within the United States during the winter season. Depending on the intensity of the snow events, deicing amounts vary from year to year. The introduced sodium chloride from deicers causes stress within nearby stream communities, especially to the periphyton community, which is the basis of in-stream primary productivity during the winter season. To determine the salinity stress within the periphyton community, I employed pulsed amplitude modulated (PAM) fluorometry. During the 2016-2017 winter season, I measured the photosynthetic efficiencies of a natural lotic periphyton community within Carroll Creek in Frederick City, MD by monitoring community stress levels under various short-term laboratory salt exposures. Laboratory test results show a short-term negative impact to the photosynthetic ability of periphyton, with an increase in sodium chloride concentration. In addition, exposing the salinity stressed samples to fresh water marked a recovery in photosynthetic ability. Further research will be necessary to establish if there are long-term effects of increased salt concentrations within Carroll Creek in Frederick City.

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# INTRODUCTION

## Background

In the late 1930s, New England states started the use of deicers on roadways to clear snow and ice. The popularity of road salt spread across the United States, with its annual use increasing from approximately 10,000 pounds in the early 1940s to approximately 15 million tons in the present day (Kelly et al., 2010). A logical solution to the over-salting of roadways is to lower the quantities of road salt in circulation. However, there is a lag time between placing salt on the roads and its presence within the groundwater systems of the Chesapeake watershed (Stranko et al., 2013).

Throughout the Northeast, snow is an ever-present element that municipalities must contend with every winter season. Like most states, Maryland is one of the many northern areas that utilize road salts in preparation for impending bad weather. The Maryland Department of the Environmental (MDE, 2015) acknowledges that the anthropogenic salt input into the environment every winter is possibly having a negative effect on local ecosystems.

## Community Development

According to Cooper et al. (2014), road salts persist in streams and eventually accumulate in the groundwater far after the time of application, even lasting into June through August. Scientists at the EPA focused on Minebank Run, which is part of Baltimore County, Maryland. The creek is located downstream of Route I-695, one of the major highway systems in the watershed. Even in summer, chloride and sodium levels are extremely elevated, suggesting that urban areas show a greater affinity to hold onto road salt contamination than more rural areas (Cooper et al., 2014). Rural sites that exhibit low population density and fewer roadways possess lower amounts of chloride ion input into nearby freshwater systems (Kaushal et al., 2005). In their experiment, various habitat types including forested, agriculture, urban, and suburban areas were considered. Within the ever-growing urban and suburban area of the Chesapeake Bay, Kaushal et al., (2005) demonstrated that chloride levels have reached the point that are known to be detrimental to the health of freshwater wildlife. Reported chloride levels of 250 mg/l are known to be harmful, with cases as low as 30 mg/l having a negative impact on plants near salt treated roadways (Table 1).

| Station | Land Use | Drainage Area (ha) | Population (Density people per ha) | Maximum Cl concentration (mg/liter) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Winter | Spring | Summer | Fall |
| With roadways | | | | | | | |
| Baisman Run | Suburban/forest (Approx. 1% impervious surface) | 381 | 1 | 38-116 | 19-29 | 22-37 | 23-29 |
| Gwynnbrook | Suburbanizing | 1,066 | 16 | 181-1,051 | 34-57 | 30-216 | 24-33 |
| Glyndon | Suburban | 81 | 9 | 229-1,509 | 79-117 | 96-469 | 72-606 |
| Villa Nova | Suburban/urban | 8,348 | 12 | 341-2,458 | 45-285 | 38-54 | 39-55 |
| Dead Run | Suburban/urban | 1,414 | 13 | 1,786-4,629 | 249-336 | 176-211 | 101-391 |
| Carroll Park | Urban | 16,278 | 20 | 960-2,085 | 63-86 | 44-86 | 49-66 |
| Without roadways | | | | | | | |
| Pond Branch | Forested | 32.3 | 0 | 3-6 | 3-8 | 3-4 | 2-3 |
| McDonogh | Agriculture | 7.8 | 0 | 5-8 | 4-5 | 4-5 | 5-7 |

Table 1: Land use and peak chloride concentrations in streams in Baltimore from Kaushal et al. (2005).

While inorganic road salts separate after dissolving into sodium and chloride ions, chloride ($Cl^-$) is not easily broken down or altered in a way that dilutes its toxicity after being introduced to a local system (Hinds and Relyea, 2019). According to the USEPA, the agency's chronic threshold for chloride ions is 230 mg/L (USEPA, 1988). Within a river or stream ecosystem, chloride ions can naturally range from 1 to 20 mg/L, while an influx into the system from road salt application can exponentially raise the range from 10 to 7,730 mg/L (Hinds and Relyea, 2019). Hinds and

Relyea (2019) reviewed the effects of road salt on all aspects of an ecosystem. A common theme amongst the studies was the wide array of concentrations levels of road salt that lead to a change in an ecosystem. Species level impacts amongst basal producers, such as periphyton; primary consumers, such as macroinvertebrates; and predators, such as fish were generally sub-lethal with reductions in productivity and/or growth and development. Community level impacts amongst all trophic levels showed a reduction in biodiversity, including some species that became salt tolerant.

**Composition of Salt Mixtures**

While sodium chloride does come from natural sources depending on the bedrock material in an area, the majority of measurable chloride concentrations are deposited from outside sources (MDE, 2015). In Maryland, the main cause for sodium chloride input into a system is from salt deicers that collect on impervious surfaces and are transported via water runoff after a winter thaw into lakes and streams (O'Hanlon and Rettig, 2010). As the population of Frederick County and Frederick City has increased, infrastructural development has led to increasing amounts of impermeable surfaces such as asphalt and concrete. Determining a safe salt concentration in these areas is difficult since Maryland regulatory agencies do not typically require monitoring for this form of contaminant in their water quality protocol. The categories mainly focused on were nitrogen, phosphorus, and sediments. The Chesapeake Bay Foundation points out that there are other deicing agents that could be substituted for salt, but their high cost and unknown effect on the environment makes them just as likely to impact the tributaries that enter the Chesapeake Bay (Chesapeake Bay News, 2010). After review of road salt policies in Maryland and surrounding states, the Chesapeake Bay Commission (CBC) determined that, to an increase in cost of road salt and road salt alternatives, the best way to counteract the environmental impacts is to closely monitor stormwater runoff and decrease the area of impervious surfaces (Stranko et al., 2013).

Within Frederick County alone, standard materials for application to roads incorporated into the budget each year prior to 2016 included bulk rock salt, antiskid material, small chips of stone for necessary traction, and Caliber M-1000, which is a highly refined complex carbohydrate corn derivative. The corn syrup consistency allows the salt to adhere to the asphalt and/or concrete surfaces and allows for a decrease in the use of typical road salt. According to the Frederick County Department of Highway and Facility Maintenance (2015), the byproduct mixture is less corrosive than the typical sodium chloride, which by their definition is more environmentally friendly. They say the additive lessens the amount of total rock salt making its way into the local watershed.

In 2016, Frederick County took a proactive stance on snow events and created their Salt Brine Pre-Treatment Program. The pretreatment solution is water that is heavily saturated with sodium chloride and is applied to the roadways within 72 hours before a storm event. Not only is the practice less expensive than typical road salt application, but the decreased addition of pure sodium chloride onto the roadways reduces the runoff quantity that will make its way into the local ecosystem (Frederick Dept. of Highway and Facility Maintenance, 2020).

Frederick City utilized a total of 3,450 tons of rock salt in 2014; 2,953 tons of rock salt and 150,000 pounds of deicer for use on parking decks and city streets in 2015; and the proposal for 2016 prior to field work for this experiment involved 3,000 tons of rock salt and 150,000 pounds of deicer (Frederick City, 2014 & 2015, The City of Frederick, 2015 & 2016). Frederick City and Frederick County contribute a minute fraction of the sodium chloride that makes its way into the Chesapeake Bay.

**Regulation of Road Salt Use**

In 2010, the Maryland State Highway Authorization in conjunction with the Maryland Department of the Environment implemented the Statewide Salt Management Plan after the passing of two bills (House Bill 0903, Senate Bill 0775; Maryland State Legislature 2010a,b), with a section devoted to environmental protection. Their overall goal is to minimize the usage of road salt to decrease costs within each county and city and lessen salt's environmental impact each year. The local municipalities plan to improve their practices by not storing typical deicing agents in environmentally sensitive areas, tightening reins on spill prevention and control plans of all agents, and utilizing industrial and/or commercial permits through the National Pollutant Discharge Elimination System (NPDES) for salt usage (MSHA, 2010). Within Maryland's larger municipalities, stormwater permits are issued that outline requirements to reduce road salt introduction to the environment through improved management plans and water monitoring to determine success of the restrictions (McKinney, 2019). With a push towards less salt usage, hopefully the state of Maryland can lead by example for other states in the region.

**Impacts on Stream Biota**

Understanding the connection between road salts and the reaction of the local biota in aquatic ecosystems will determine the level of stress that is present. Within a stream system, periphyton is the community of biota that forms the base of the food web and can include attached algae, fungi, plant detritus, bacteria, and other microscopic organisms. Periphyton play a major role in these systems by acting as primary producers to feed higher trophic levels. Besides food, periphyton increases dissolved oxygen through photosynthesis which allows aquatic insects and smaller biota to survive. The organisms that rely on periphyton normally are a good indication of water quality in a system (Gommermann, 2014). Streams are strongly influenced by the water flow and disturbance events within a system.  A sudden change in environmental makeup can either negatively or positively affect a system. If an increase of rock salt is present in a system, plants that are sensitive to saline levels will suffer (Hillebrand and Kahlert, 2001).

A field-based experiment involved the introduction of 1000 ppm of sodium chloride into a stream. This led to a decrease in the algal density during the last three weeks of the four-week experiment (Dickman and Gochnauer, 1978). The results of the laboratory-based Van Meter et. al. experiment (2011) showed similar findings with the prior experiment, except periphyton communities were negatively affected at lower chloride levels of 645 ppm. To expand further on the idea that the addition of road salts into an environment affects the algal community, Van Meter et al results showed food webs in affected stream systems would be altered. Results suggest that road salt directly eliminates zooplankton from the ecosystem. The depletion of a competitor allows primary producers to thrive and tadpoles indirectly benefit from the absence of zooplankton. Lastly, Cochero et al. (2017) report that the stress caused by salt treatment on various primary producers was diminished after 72 hours. They were able to recover back to normal conditions after the treatments had been withdrawn for the same amount of time. This experiment demonstrates that salt introduction into a system may be recoverable when introduced as a temporary stress.

The purpose of my experiment is to determine whether road salt application has an effect on local ecosystems within Frederick City, Maryland. The two hypotheses tested in this experiment are 1) A natural harvested periphyton community's photosystem II levels will decrease when exposed to salt treatment solutions of 4 ppt, 6 ppt, and 11 ppt and; 2) A natural harvested periphyton community's photosystem II levels will recover when introduced to freshwater after exposure to salt treatment solutions of 4 ppt, 6 ppt, and 11 ppt.

## MATERIALS AND METHODS

The experiment took place in a portion of Carroll Creek within Baker Park, a tributary of the Monocracy River in Frederick, Maryland (Figure 1).

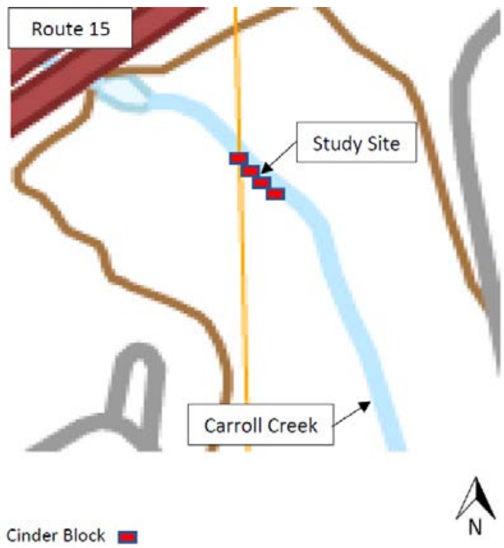


Figure 1: View of experiment site along Carroll Creek within Baker Park in Frederick City, Maryland.

The field portion of the experiment began at the end of November 2016 and finished in early February 2017. Four cinderblocks with a dimension of 4’’x 8’’x 16’’ had eight 2’’x 2’’ standard shower tiles adhered to the cinder blocks’ surface with a silicon adhesive. Each cinderblock was held in place with metal cables that were anchored to an exposed tree root along the side of the stream channel. Periphyton grew in mats on the shower tiles for ten weeks. Each week, the experiment site was visited to monitor the growth of periphyton on the tiles. After sufficient growth was present, the cinderblocks were removed from the stream and all tiles were taken from the cinderblocks and placed within a clear storage tote filled with freshwater from the experiment site. While in the field, stream water was collected in clean plastic jugs and transferred to the lab for use in the remaining portion of the experiment.

All 32 tiles were placed in individual petri dishes with an opaque bottom and clear top filled with water collected from the experiment site. The tiles were stored at 5 °C with a constant light source. The clear top had a puncture hole large enough to later insert the fiber optic tube of the PAM fluorometer (Junior-PAM, Heinz Waltz GmbH, Effeltrich, Germany) to obtain a reading. Thirty minutes prior to the first reading to establish a baseline measure of Photosynthesis II efficiency in each periphyton mat, a blacked out petri dish covered the sample. When it was time for the first reading, I replaced the dark cover with the clear cover with the puncture hole. During the base reading, four data points were randomly selected to establish an average maximum photosystem II efficiency reading on each tile.

Prior to preparation of the salt treatments, the collected water from the experiment site was filtered through a 0.8 μm pore-sized disposal filter held by a Nalgene Rapid-Flow filter unit and was attached to a manual vacuum pump. After filtration, a refractometer was used to determine that the initial salinity of the filtered creek water was 1 ppt.

The stream water collected in the field was used to create four treatments: 1) the control was 1 ppt filtered water unaltered from the field; 2) filtered stream water treated with NaCl to produce a 4 ppt solution; 3) a 6 ppt solution; and 4) a 11 ppt solution.

To easily analyze if salinity affects a periphyton community, it is common to utilize pulse amplitude modulation, or PAM, fluorometry. Within an autotrophic organism, light energy is absorbed and utilized by the chlorophyll molecules to promote the excitation of an electron to a higher energy level. After an excitation event, between 2 and 10% of absorbed light is radiated as photosystem II fluorescence, which indicates the efficiency performance of a photosynthetic organism.  When measuring the PS II activity, the PAM fluorimeter interprets its findings on a scale between 0.02 and 0.1. The lower the fluorescence measurement, the lower the photosynthesis efficiency of an organism (Walz, 2007). This technology allows researchers to noninvasively and quickly obtain extensive information about the efficiency of a photosynthesizing organism (Schreiber 2004). Utilizing this tool is common amongst physiological studies and is beneficial when determining the amount of stress that salinity has in a stream system.

Two of the eight tiles from each of the four cinder blocks were placed in each of the four treatments. The tiles in each petri dish were immersed in the assigned treatment and kept at 5 °C for 1 hour. Thirty minutes prior to the first reading after the treatments were applied, a blacked out cover was replaced with the clear cover to once again acclimate each sample for a reading. At the one-hour mark, just like the baseline reading, four data points were taken to establish an average maximum photosystem II efficiency reading per tile. This process was repeated to obtain readings at 24, 48, 72, and 96 hours.

After the results of the 96-hour period were collected, a second incubation was conducted to determine if the periphyton communities could recover after being exposed to elevated salinity. within a system. All salt solutions were replaced with filtered stream water. Again, PAM readings were taken at 1, 24, and 96 hours, similar to the NaCl treatments. After the 96 hours readings, all treatments were set aside. Three percent hydrogen peroxide was added to all controls but one. After incubation for an hour, no control samples responded to PAM fluorometry, while the remaining control sample continued to respond to fluorometry similarly to its 96 hour reading.

Statistical analysis on the resulting data from the field and laboratory measurements was completed using Excel to assess potential differences in the maximum photosystem II efficiency amongst the periphyton exposed to four NaCl treatments. Results were statistically analyzed utilizing a one-way analysis of variance (ANOVA) to determine if there are statistically significant differences between the salt treatments. A Tukey's Post Hoc test was applied to the results that were shown to be statistically significant through the ANOVA to determine where the significance is within the data. The Tukey Test is used when the sample sizes for each level are not equal.

## RESULTS

### The Effect of Short-term Salinity Stress

Short-term salinity stress causes photosynthetic efficiency in stream periphyton to decrease. The average PSII efficiency level of each sample after the initial exposure to treatments 4 ppt, 6 ppt, and 11 ppt remained relatively constant until a noteworthy decrease after 72 hours with output leveling off at 96 hours (Figure 2).

Several one-way analysis of variance tests (ANOVA) were conducted to compare the effect of salt treatments on natural harvested periphyton communities in 4 ppt, 6 ppt, and 11 ppt conditions at different time points. An ANOVA was nonsignificant between treatments at 1 hour, 24 hours, and 48 hours, but there was a significant different between treatments at 72 hours ($F(3,19)=3.31$, $p=0.04$) and 96 hours ($F(3,19)=20.26$, $p=3.84E^{-06}$).

Figure 2 shows the variance amongst the average initial PS II efficiency readings per tile per treatment over time. The standard error bars between treatment levels heavily overlap within 0 through 48 hours, which means there is no statistical difference between the means, but they begin to spread out within 72 through 96 hours showing a statistical difference between the means. There are significant differences between each treatment level after 72 hours. There was a minor increase in PSII readings for the control at 96 hours; however, the laboratory manipulation over time decreased the vitality of the periphyton. The slight increase does not demonstrate a biological increase of photosynthetic ability. Figure 2 displays the initial sample size at each time period, starting with the baseline reading until the last treatment reading at 96 hours. The experiment began with 32 tiles with four per treatment type. The reason sample sizes change over time is because one tile for the control and one tile for the 4 ppt, and three tiles for the 6 ppt and for the 11 ppt treatments did not have a measurable PS II reading after 100 attempts using the PAM Fluorometer. These tiles were discarded from the entire experiment. As the experiment continued, only one tile was lost for the 4 ppt treatment at 24 hours. Every other tile after the initial treatment remained because a PS II reading was detected showing photosynthesis efficiency. When a significant result from an ANOVA was found between treatment times, a Tukey's Test was performed to determined where the difference amongst the means was notated in Figure 2.

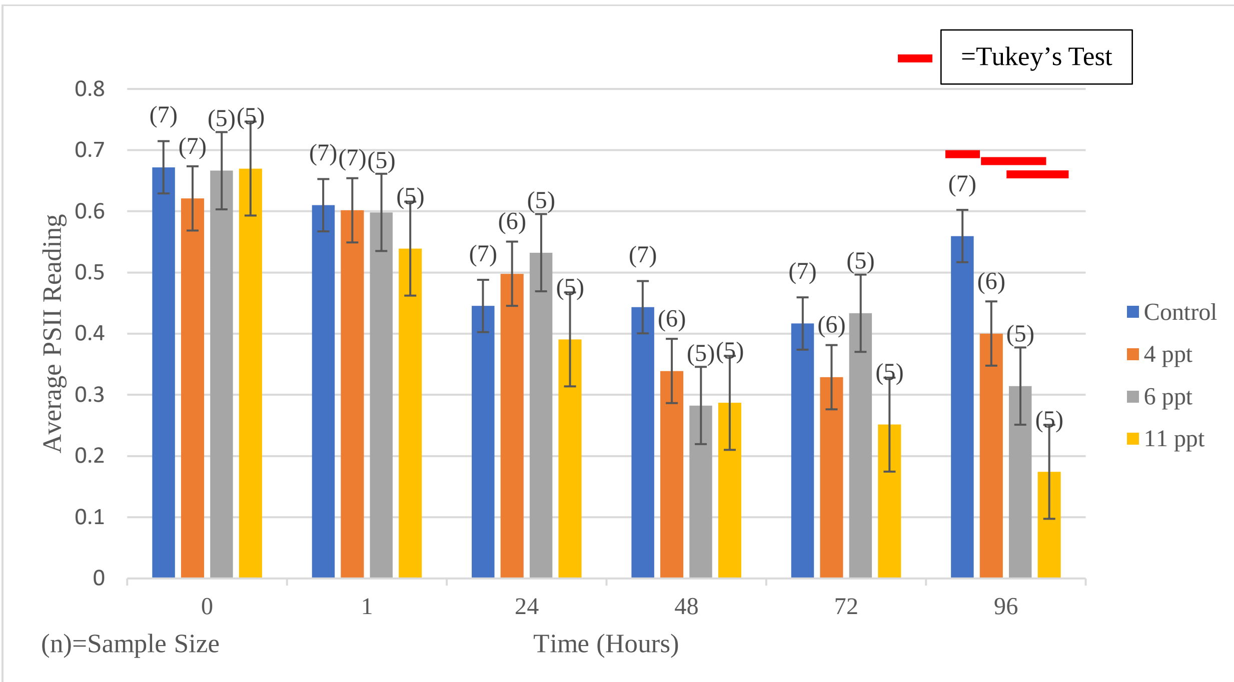


Figure 2: Initial average PS II efficiency readings across four points on tiles all exposed to treatment is observed in this bar graph. Seventy-two hours had a $p<0.05$, but the Tukey Test results deemed insignificant relations between the means. The horizonal red lines above the treatments at 96 hours visually demonstrates the Tukey's Test results where the differences amongst the means are expressed. Within 96 hours, the control is statistically different than every other treatment. As expected, the control did not show a significant change over time from time zero through 96 hours based on no manipulation to the stream water. Treatments 4 ppt and 6 ppt are statistically the same and treatments 6 ppt and 11 ppt are statistically the same. Treatments 4 ppt and 11 ppt have a statistical difference amongst their means which is why no bar overlaps all 4 ppt, 6 ppt, and 11 ppt.

## Recovery from Salinity Stress

Exposure to freshwater does increase photosynthetic efficiency in stream periphyton after a short-term salinity stress event. The average PS II efficiency of each short-term stressed sample after exposure to fresh water remains relatively constant at 4 ppt and 6 ppt with a noteworthy increase at 11 ppt. There was a notable decrease in the control at 96 hours however, the laboratory manipulation may have negatively affected the sample (Figure 3).

Several one-way analysis of variance tests (ANOVA) were conducted to compare the effect of freshwater on natural harvested periphyton communities after exposure to 4 ppt, 6 ppt, and 11 ppt conditions through time. An ANOVA was significant across time for the control ($F(3,24)=4.12$, $p=0.01$) and 11 ppt ($F(3,16)=11.97$, $p=2.31E^{-04}$), but an ANOVA was nonsignificant through time for 4 ppt and 6 ppt.

Figure 3 shows the variance amongst the average PS II level readings per tile per treatment through time. There are statistical differences through time within the control and 11 ppt, but not within 4 ppt and 6 ppt. The standard error bars overlap within 4 ppt and 6 ppt, while the standard error bars, except at 96 hours overlap within the control and 11 ppt. The standard error bars within the control all heavily overlap, except at the 96 hour mark meaning the means within the treatment are similar between times 1 hour, 24 hours, and 48 hours; but there is a difference amongst the means at 96 hours. The standard error bars within the 4 ppt and the 6 ppt treatments heavily overlap, indicating the means within each treatment are similar through time. According to the ANOVA test for 4 ppt and for 6 ppt, there is no difference within the means of those treatments. There is a notable separation of the standard error values from treatments 4 ppt and 6 ppt, but there is no statistical difference found through the ANOVA test. Lastly, the standard error bars within the 11 ppt treatment are further spread apart than the 6 ppt and the significant difference amongst the means was determined during the ANOVA test. The PS II level for 11 ppt at 96 hours is the furthest from the other PS II levels than in every other treatment. Figure 3 displays the recovery sample size at each time period, starting with hour 1 of the periphyton being accustomed to the dark, until the last reading at 96 hours within each treatment. There is no hour zero for the recovery sample size because it is the 96 hour sample size in the initial treatment readings over time. Readings occur at hour 1 after acclimation to the dark. There was a minor decrease in PSII readings for the control through time, which is possible after 192 hours of laboratory manipulation spread across two experiments probably decreased the vitality of the periphyton. This section of the experiment began with 23 tiles with various sample sizes within each treatment through time. As the recovery experiment continued, no tiles were lost. When a significant result from an ANOVA was found within each treatment, a Tukey's Test was performed to determined where the difference amongst the means was notated within Figure 3.

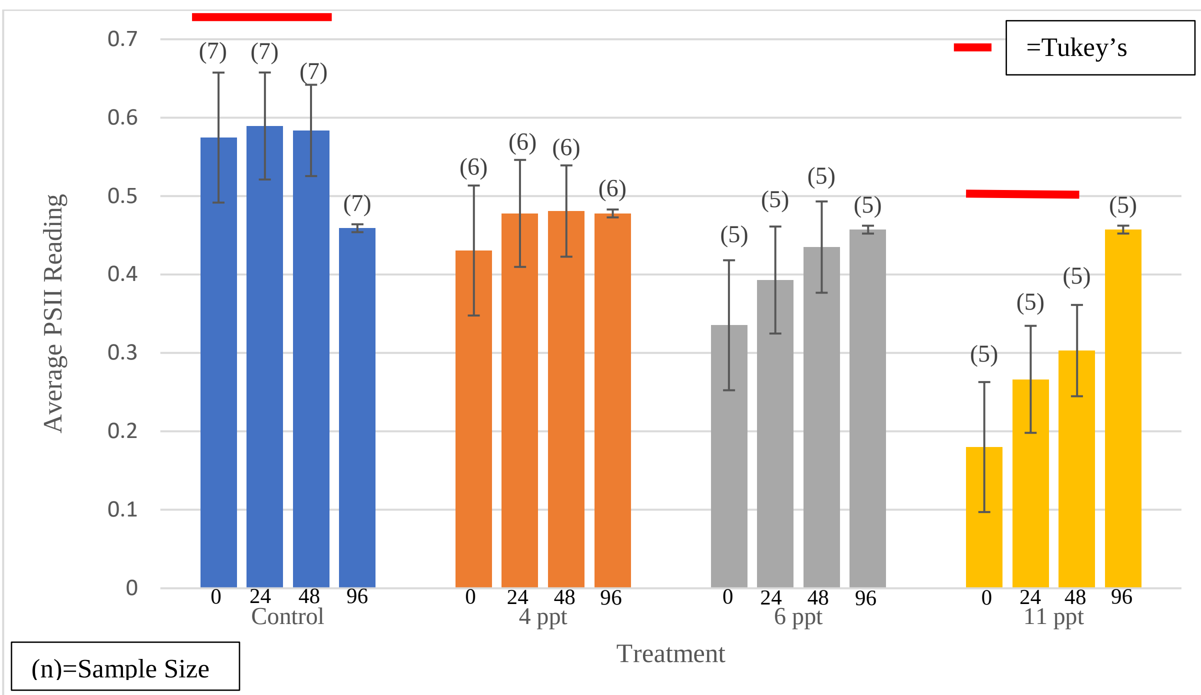


Figure 3: Average recovery PS II readings across four points on tiles exposed to freshwater after exposure to short term salinity stress. The horizonal red lines above the control and 11 ppt visually demonstrates the Tukey's Test results where the differences amongst the means are expressed. The control in relation to itself through time at 0 hour, 24 hours, 48 hours, and 96 hours show no statistical difference through time; except between 24 hours and 96 hours and 48 hours and 96 hours. Treatment 11 ppt is statistically the same at 0 hour, 24 hours, and 48 hours, while 11 ppt is statistically different through time at 96 hours.

## DISCUSSION

I performed this experiment to discover the short-term effects of salinity stress on the photosynthetic ability of microscopic stream biota to assess potential biological effects from salt treated roadways into Carroll Creek within Frederick, Maryland. This was accomplished by exposing a naturally-derived periphyton community to simulated salting after a snow event within a laboratory setting.

In the first experiment, I tested if a natural harvested periphyton community would be affected by salt treatment solutions of 4 ppt, 6 ppt, and 11 ppt. Photosynthetic efficiency showed a decline when exposed to salt treatments, especially by 96 hours. Like in my experiment, Dalinksy et al. (2014) mimicked aquatic stressors, such as road salt impact within a laboratory setting. Road salt impact was simulated by creating solutions between 1 and 3,000 ppm. This experiment simulated conditions of freshwater (anything under 1 ppt) to 3 ppt, which is lower than my experiment with increased exposure up to 11 ppt. In their results, periphyton biomass prospered from a rise in salt concentration. I did not measure periphyton biomass within my experiment, but there was a notable decrease of available periphyton based upon the removal of nine tiles due to algae demise prior to data collection, but after exposure to the salt solutions. The exposure of the NaCl may not have been the initial factor for increased mortally and instead, the periphyton within a laboratory setting or petri dish, and not within a natural body of water, may have negatively affected the health of our algae. As shown within Figure 2, the control slightly decreased in PSII levels over time which does support this idea based upon no salinity manipulation to the control. Even so, increased salinity tends to result in a decline in native biodiversity (Dalinksy et al. 2014).

In relation to our decreased photosystem II (PSII) values as a result of increased salinity, Cook and Francoeur (2015) also showed exposure to salt-amended stream water significantly reduced PSII levels of periphyton over their 24 hour experimental period. Cook and Francoeur

(2015) also used PAM fluorometry as their indication of photosynthetic success. In experiment 2, I tested if a natural harvested periphyton community would recover when introduced to freshwater after exposure to salt treatment solutions of 4 ppt, 6 ppt, and 11 ppt. When samples originally treated with salt solutions were introduced to stream water from the field, photosynthetic efficiently increased, specifically with periphyton originally exposed to 11 ppt. Cook and Francoeur (2015) took a similar approach to see if salinity stressed communities could recover, and their results were similar to mine. In their experiment and my own, Photosystem II yield in freshwater and low salinity treatments increased slightly, but was not statistically different. The photosystem II yield of the high salinity treatment at 35 ppt increased significantly for Cook and Francoeur (2015). The photosystem II yield of our highest salinity treatment at 11 ppt increased significantly through time. Their high salinity treatment is the same as ocean water and we kept our treatments lower to simulate a realistic reading within freshwater in the field after a storm event.

Within Figure 3, the control showed a decrease in PSII output after 96 hours. In our case, the naturally grown periphyton may have been stressed from not being in a natural, oxygen rich environment. Another item to note is the freshwater collection from the stream was already at 1 ppt, since a storm event occurred during the periphyton growth time period. Our control was still considered freshwater, but the elevated salinity within the stream may have increased the tolerance of our periphyton and yielded higher PSII readings within the laboratory. Naturally elevated salinity levels within our stream demonstrate the extended length of time salt remains after a winter storm event.

Costello et al., (2018) determined that amongst all types of stressors in the Huron River system in Michigan, periphyton was least likely affected by salt. Their reasoning was since climate change and rising temperatures lessen the possibility for snowfall, road salt application will decrease. On the contrary, Stirpe et al., (2017) examined the potential outcomes of climate change on rates of salt transfer from watersheds and projected that less winter storms from rising temperatures will increase rainfall through tropical storms, and road salt will be removed faster from an ecosystem. Either way, both studies determined that climate change will inevitably decrease salt concentrations within a stream system. According to Espinola et al., (2014), there is a potential for Frederick, Maryland to experience a projected temperature increase of 1.8 °F by 2025 and 5.4 °F by 2100. Research from Syracuse University using climate modelling predicts that by 2050, reduced snowfall due to increased temperatures will lessen the yearly anthropogenic sodium chloride introduction into an ecosystem (Gutchess 2018). In the future, periphyton may benefit from climate change because of decreased salinity induced stress to an ecosystem.

This research demonstrates short-term salt exposure directly caused reductions in photosynthesis efficiency in stream periphyton in Carroll Creek. The stress induced by salinity introduction was reversible by exposing the short-term stressed samples to freshwater retrieved from the field. Laboratory induced short-term salt introductions do not appear to cause lasting damage to local periphyton in Frederick, Maryland, however, further studies are necessary to determine the long term effects of road salt within the local area.

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