5-2023

Investigating Stormwater Parameters from Runoff on East Tennessee State University Campus

Abby McIver
East Tennessee State University

Dr. Joseph R. Bidwell
East Tennessee State University

Investigating Stormwater Parameters from Runoff on East Tennessee State University Campus

By

Abby McIver

An Undergraduate Thesis Submitted in Partial Fulfillment

of the Requirements for the University Honors in Discipline Program

East Tennessee State University

Abby McIver

Abby McIver

Joe Bidwell

Dr. Joseph R. Bidwell, Thesis Mentor

Trevor Chapman

Dr. Trevor L. Chapman, Reader

ABSTRACT

Climate change has caused an increase in extreme rain events and flooding in certain regions across the globe. During rain events, water flows over impervious surfaces structures such as roads and sidewalks, picking up contaminants such as metals, fertilizers and other nutrients, and various organics that which may impact organisms in such as streams, river, and lakes. Previous work has found significant differences in survival of organisms that were exposed to contaminated stormwater runoff. This study investigated stormwater chemistry parameters at collection sites on the East Tennessee State University campus. Sites were selected based on the extent of human interaction and traffic in the areas. Additionally, acute toxicity of stormwater samples was investigated through 48-h bioassays with the cladoceran, *Daphnia magna*. In September and November 2022, water chemistry and toxicity analyses were conducted across multiple rain events and over a six-hour time course of an individual rain event. For each of the events and the time course, chlorophyll levels, specific conductivity, pH, temperature, and dissolved oxygen were measured. No statistical difference between the water chemistry parameters between sampling sites or between rain events were observed. Additionally, no significant differences in 48-h survival of *D. magna* were detected between sampling locations or during the single event time course study. These data suggest that there were no pollutant surges at the collection sites and that *D. manga* survival was not affected by the contaminants.

ACKNOWLEDGEMENTS

I would first like to thank my mentor Dr. Joe Bidwell for taking a chance on me and pushing me to be a better student and scientist. I would also like to thank my reader and friend Dr. Trevor Chapman for his guidance and patience throughout this process. Finally, I would like to acknowledge my parents Gary McIver and Christy Harpine as well as my close friend Anna Grace Grizzard for their continuous support and words of encouragement. Each of you have encouraged me to grow personally and professionally and completing this project would not have been possible without each of you. Thank you all.

TABLE OF CONTENTS

CHATPER 1: INTRODUCTION

Stormwater

In recent years, there has been an increase in rain events throughout the world causing major flooding and damage to landscapes (Post & Knapp, 2020). As a result, interest in better understanding the potential impacts of stormwater runoff on aquatic ecosystems has grown. Studies have shown that stormwater runoff in residential and commercial areas contained pollutants such as pesticides, heavy metals, fertilizers, and hydrocarbons (Burant, et al., 2018; Brown et al., 2012; Young et al., 2018), all of which could impact aquatic organisms.

Land Use

Studies have shown that differences in land use influence chemical composition of stormwater runoff (Sajikumar & Remya, 2015). For example, runoff collected from commercial and industrial sites often contains high levels of contaminants as compared to that from rural areas (Sajikumar & Remya, 2015). Urban areas have higher human populations and more impervious areas such as roadways and parking lots which cause more pollutants such as metals, hydrocarbons, and pesticides to accumulate. These toxicants can be picked up by runoff during rain events and enter the surrounding ecosystems (Brown et al., 2012). Although rural areas have lower human populations and urban runoff, other factors such as livestock, pesticide use, and agriculture have potential to contaminate the surrounding ecosystems as well. One study examined agricultural stormwater runoff for contaminants such as pesticides, insect repellent, and industrial chemicals. A nearby stream affected by the agricultural runoff was tested and 17 of the 31 total contaminants were found (Tran et al., 2019). Studies have found that roads and highways are another example of land use that contaminate stormwater runoff (Brown et al., 2012; Butler et al., 2015; Kim et al., 2005; Marsalek et al., 1999). Due to the impervious nature of highways pollutants such as oils, grease, fuels, heavy metals, road salts and more can accumulate. It was found that runoff from the first flush or initial surface runoff of a rain event collected most of these pollutants and contaminated the surrounding areas (Kim et al., 2005).

Aquatic and semi-aquatic habitats are being contaminated with chemicals collected by runoff and thus affecting the health of the organisms living in the area. Frogs, fish, and invertebrates have all been used as test species for acute stormwater runoff toxicity tests. One study examined stormwater parameters as well as their effects on fish, *Pimephales promelas* and *Ceriodaphnia dubia* in stormwater runoff on a college campus (McQueen et al., 2010). The results suggested that fish were extremely sensitive to the contaminants and had a significant decrease in survival after exposure. However, the *C. dubia* only experienced high mortalities after being exposed to a sample of first flush stormwater runoff (McQueen et al., 2010). Amphibians have also been used in studies as tests organisms to demonstrate the effects of stormwater runoff and its contaminants on survival and development. For example, imidacloprid, a pesticide, was lethal to tadpoles when exposed to concentrations equivalent to what has been found in runoff (Sievers, et al., 2018). Bosisio et al. (2009) investigated the effect of sodium chromate which is found in petroleum on *X. laevis* embryo development. Sodium chromate is a common contaminant found in stormwater runoff from highways experiencing high vehicular traffic. They found extreme deformities including coiling of the tail, body oedema, and stunted growth after exposure to every concentration of the toxin. Another study found that sodium chloride, a common toxicant in road salt, was extremely lethal to both Cope’s gray tree frog, *Hyla chrysoscelis* and the green frog, *Rana clamitans* embryos with above 50% mortality after each exposure (Brown et al., 2012). Young et al. (2018) found that toxicants such as copper in stormwater runoff negatively affect zebrafish, *Danio rerio,* in the larval stage and salmon, *Salmo salar,* embryos by decreasing the amount of hair cells created during embryogenesis which are used to detect water movement after development (Young et al., 2018). Invertebrates have also been used as model organisms for the effects of contaminated stormwater runoff. Gardner and Royer (2010) exposed *Daphnia pulex* to different concentrations of road salt equivalent to what was found in urban runoff. A concentration level of 2500 mg/L^{-1} that is based on high road salt use during the winter months of Indiana was used. After exposure to this level of road salt *D. pulex* had complete mortality suggesting the concentration is detrimental to aquatic invertebrates (Gardner & Royer, 2010). Research such as this raises questions as to the true extent of contaminated stormwater runoff and its effects on the organisms of the surrounding ecosystems.

Objectives and Hypotheses

The objective of this study was to further investigate the effects of stormwater runoff at sites on the East Tennessee State University campus using aquatic test species. The initial plan was to compare developmental effects of stormwater collected from campus sites with different land use on embryos of the African Clawed Frog, *Xenopus laevis*. However, due to poor control survival of commercially sourced embryos, the test species was changed to the water flea, *Daphnia magna.*

It was predicted that stormwater from campus collection sites with the highest amount of vehicular traffic would be more toxic to *D. magna* as indicated by 48-h acute toxicity tests. Additionally, it was predicted that values for a suite of basic water chemistry parameters collected from the sites would differ between those with greater vehicular traffic and those without.

CHAPER 2: METHODS

Site Selection

Field sites included four locations on the East Tennessee State University campus; a storm drain behind the Culp center (Culp drain) a second stormwater drain beside the tennis courts (tennis court drain), the gutter draining a parking lot across from the Pal's restaurant (Pal's p. lot), and a gutter draining the end of a parking lot across from the parking garage (parking garage p. lot). The stormwater drain sites were selected based on a previous study conducted by faculty and students from the ETSU Department of Geosciences that indicated the potential for contaminants. The Pal's p. lot site was selected because it is influenced by an extremely busy parking lot and so potentially influenced by contaminants from cars and the parking garage p. lot was chosen because it represents an area with significant impervious surface but little vehicular traffic.

Test Organisms

Xenopus laevis was originally chosen as the model organism for the study because of their availability and sensitivity to contaminants. *Xenopus* eggs were ordered from Xenopous-I Inc (Dexter, MI) and were delivered 1-2 days after the eggs were laid.

After initial trials with *X. laevis* proved unsuccessful, the study organisms were changed to the cladoceran *Daphnia magna.* These organisms were collected from an existing culture in Department of Biology at ETSU and bioassay procedures with neonates less than 24 h old were conducted according to USEPA (EPA, Office of Water, 2002).

Water Quality Sampling and Testing

During major rain events a ProDSS handheld multiparameter sampling instrument (YSI Inc., Yellow Springs, OH) was used to measure temperature, dissolved oxygen, specific conductivity, pH, and chlorophyll. A 2000 mL sample of stormwater was also collected from each site in a plastic 7580 mL container and returned to the laboratory after collection in an air-conditioned vehicle. Roughly 30 minutes after collection, analysis of nitrate and phosphate levels using Hach water test kits (Hach Company, Loveland, CO) were completed. Detection levels were above 0.01 for both nitrate and phosphate.

Water quality samples were taken from September 2022 to November 2022. The first recording was completed on September 9th 2022, the second was September 30th, the third October 16th, and the last sampling event was November 11th. The last rain event recorded (November 11th) had multiple sampling events to investigate any changes in water quality parameters or toxicity over a time course during the rain event. These samples were taken every two hours from the start of the rain event allowing one hour for field sampling and collection at each site.

Toxicity Test Procedure

Once the *Xenopus laevis* eggs were delivered, they were immediately dejellied by stirring in a 2% L-cysteine solution for two minutes (ATSM, 1991). The eggs were then moved to 30 mL petri dishes containing 25mL of FETAX solution (ATSM, 1991) with 25 eggs per petri dish. After staging per FETAX guidelines, the eggs were placed in a ThermoScientific refrigerated incubator (Sterilelink Inc., Greensboro, NC) at 24°C ± 2°C for 96 hours. Every 24 hours during the test, dead embryos were identified by visible deterioration of the outer envelope and then removed from the petri dishes while the surviving embryos were placed back in the incubator for the remainder of the test. When the test was complete at 96 hours the embryos were investigated under a Leica MZ 9.5 dissecting microscope (Martin Microscope Company, Easley, SC) for any mutations. The embryos reached up to stage 40 of development and were euthanized using a 20% tricaine solution buffered with sodium bicarbonate (ATSM, 1991).

Toxicology tests for *D. magna* were completed via EPA guidelines (EPA, Office of Water, 2002). The standards called for a toxicity test that was 48 hours in duration during a normal light cycle. Organisms were to be held in a 30 mL petri dish filled with 25 mL of solution. Tap water was boiled on a stove for 20 minutes to make dechlorinated tap water which was used as a control. Following EPA guidelines, four replicate tests were completed each exposing five *D. magna* to a sample (EPA, Office of Water, 2002). A total of twenty *D. magna* were exposed to samples from Pal's Lot (S), Parking Garage Lot (S), Culp Drain (S), and Tennis Court Drain (S). Due to a lack of *D. magna* under 24 hours old, the control and samples taken at the beginning of the rain event were prioritized and received five *D. magna* per four replicates. Although five *D. magna* were still exposed in a replication, the replications were limited for the remaining samples. *D. magna* were divided amongst the remaining samples allowing only ten to be exposed to the Culp Drain (F) and Tennis Court Drain (F) samples, nine to be exposed to the Parking Garage Lot (F) sample, and five *D. magna* to be exposed to the Pal's Lot (F) sample. After the end of the 48-hour test, the surviving *D. magna* were euthanized in a 70% ethanol solution.

Data Analysis

Statistical analyses were conducted using the replicate measurements of water quality variables collected during the time course study and on survival of *D. magna* exposed to stormwater samples from each of the collection sites. Data were first analyzed for normality and equality of variance and a one-way analysis of variance (ANOVA) was used to compare the data between sites. If significant differences between sites were detected, a Tukey post-hoc test was used to compare values from individual sites. All analyses were conducted at α=0.05 using Sigmaplot Version 14.5 (Systat Software, Inc, San Jose, CA).

CHAPER 3: RESULTS

X. laevis

Three separate trials with *X. laevis* were conducted to evaluate survival of the embryos received from the supplier. Unfortunately, survival of embryos under control conditions was consistently low therefore the decision was made to use *D. magna* as the test organism in the study.

D. magna

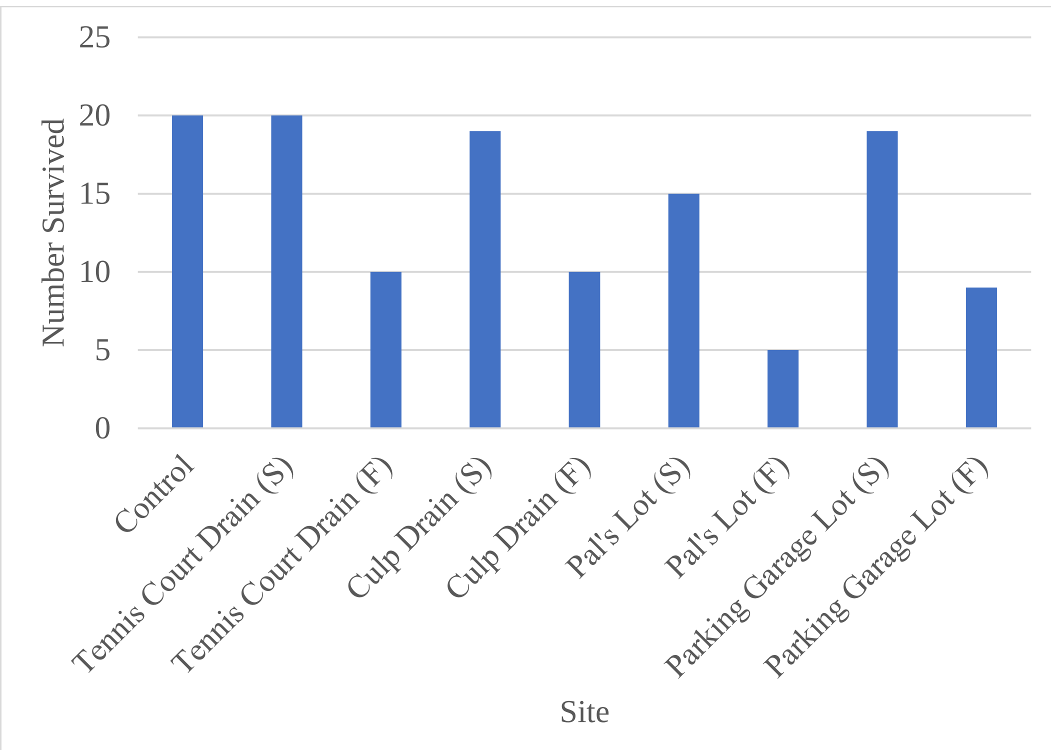

Figure 1. Total number of *D. magna* (n=20) survived after exposure to water samples collected at the beginning (S) and end (F) of a rain event on 11/11/2023 at each collection site.

The total number of *D. magna* that survived exposure to each water sample collected during a time course rain event is shown in Figure 1. Of the beginning (S) rain samples (n=20) collected the Tennis Court Drain (S) site was the only to have full survival while the Culp Drain

(S), Pal's Lot (S), and Parking Garage Lot (S) had mortalities of 1, 5, and 4 individuals respectively. The Tennis Court Drain (F) (n=10), Culp Drain (F) (n=10), Pal's Lot (F) (n=5), and Parking Garage Lot (F) (n=9) sites had no mortalities.

Storm Water Testing

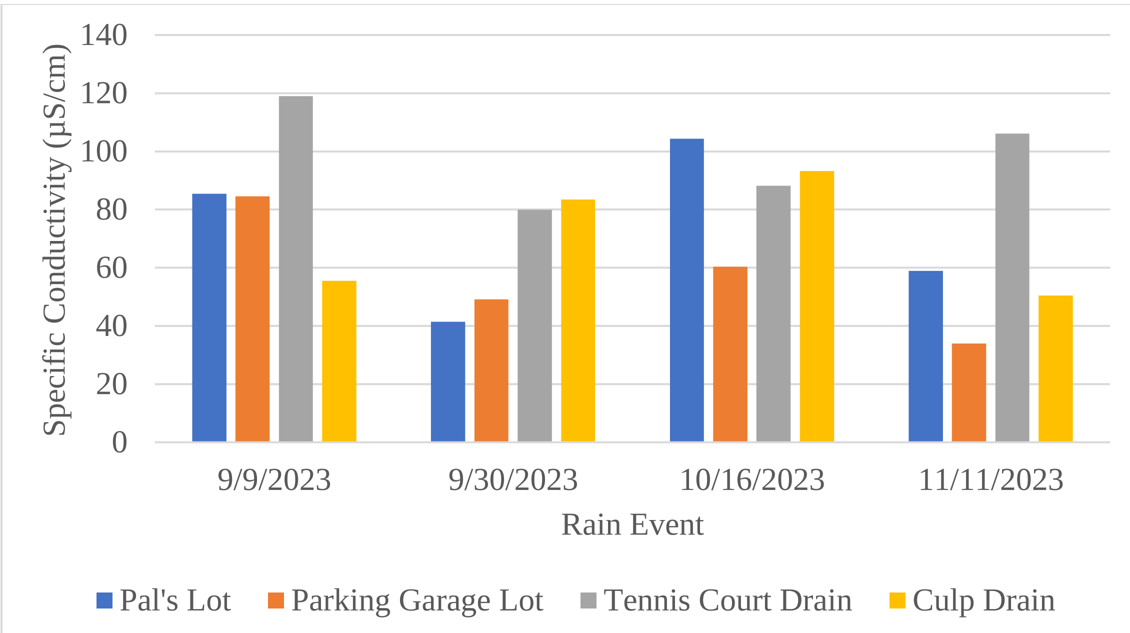

Figure 2. Individual specific conductivity measurements at each collection site during the four rain events sampled.

Specific conductivity measurements recorded at collection sites on 9/9/2023, 9/30/2023, 10/16/2023, and 11/11/2023 showed an amount of variation with no evident trends (Fig. 2). Specific conductivity collected during the 9/9/2023 rain event had the highest level, 119 µS/cm, at the Tennis Court Drain collection site and the lowest level, 55.5 µS/cm, at the Culp Drain. There was little consistency in conductivity across sites and rain events. For example, the lowest conductivity value for the 9/30/2023 event was 41.1 µS/cm at the Pal's Lot site while the highest was 83.4 µS/cm and observed at the Culp drain. The 10/16/2023 event had its highest measurement, 104 µS/cm, at the Pal's Lot while the Parking Garage Lot was the lowest measurement recorded during the event at 60.3 µS/cm. Like the 9/9/2023 rain event, the 11/11/2023 event had a higher conductivity level at the Tennis Court Drain, 106 µS/cm, however, the lowest measurement of the event was 34.0 µS/cm recorded at the Parking Garage Lot.

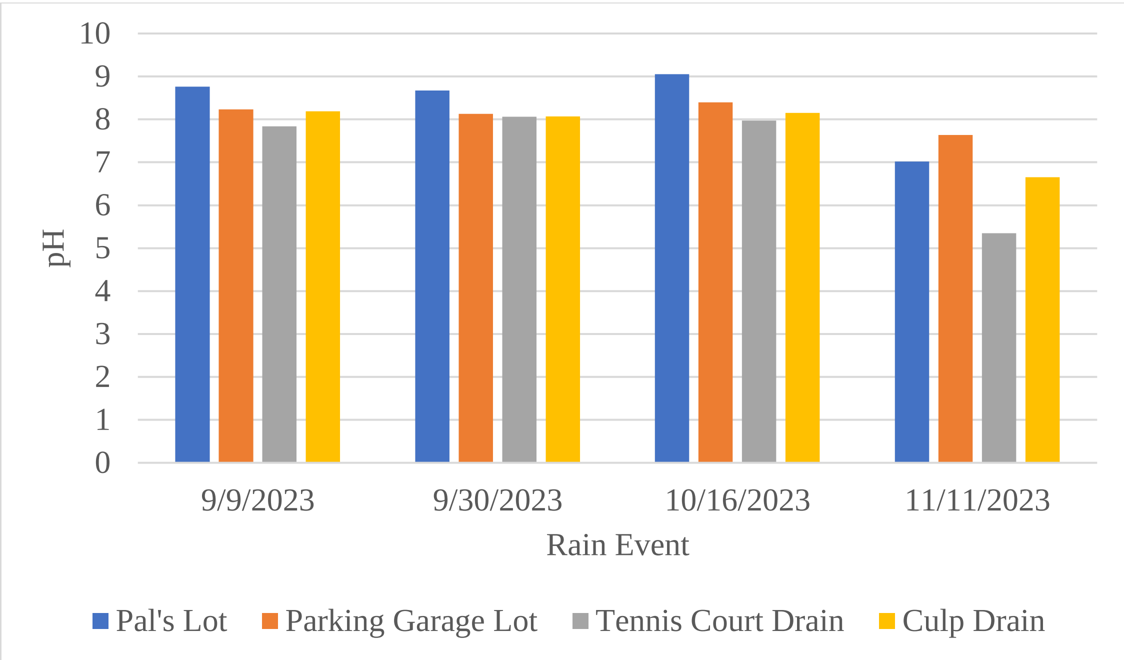

Figure 3. Individual pH measurements at each collection site during the four rain events sampled.

Overall, the pH at each site was slightly above neutral. Rain events on 9/9/2023, 9/30/2023, and 10/16/2023 had a similar trend regarding the pH with the Pal's Lot being the highest while the other sites maintained almost the same pH level over the three recordings which was slightly lower than the Pal's Lot measurements. The rain event on 11/11/2023 had a reduced pH for all the sites, with the Tennis Court Drain having the lowest level of all. The data collected at the other three sites were still close to neutral but lower than measurements recorded during other rain events.

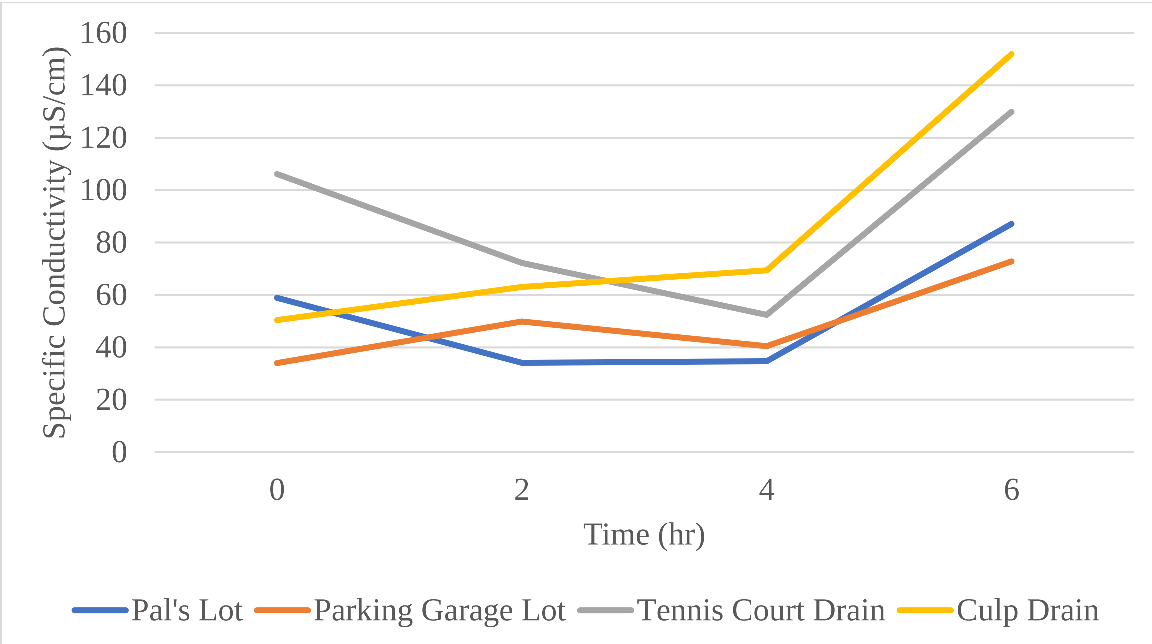

Figure 4. Individual specific conductivity measurements collected every two hours over a six-hour rain event on 11/11/2023.

Conductivity varied over the course of the rain event at each of the sampling sites, although some general trends were apparent as shown in Figure 4. Both the Tennis Court Drain and Pal's Lot decreased over the first two hours of the event. Conductivity in the sample from the Pal's Lot then leveled off between hours 2 and 4, while those levels from the Tennis Court Drain continued to decline until hour 4. Conductivity values in the Culp Drain samples exhibited a steady increase from the start of the event through hour 4, while values in the samples from the Parking Garage Lot increased from the start of the event to hour 2 and then declined slightly between hours 2 and 4. Interestingly, conductivity values at all sites exhibited a sharp increase between hours 4 and 6.

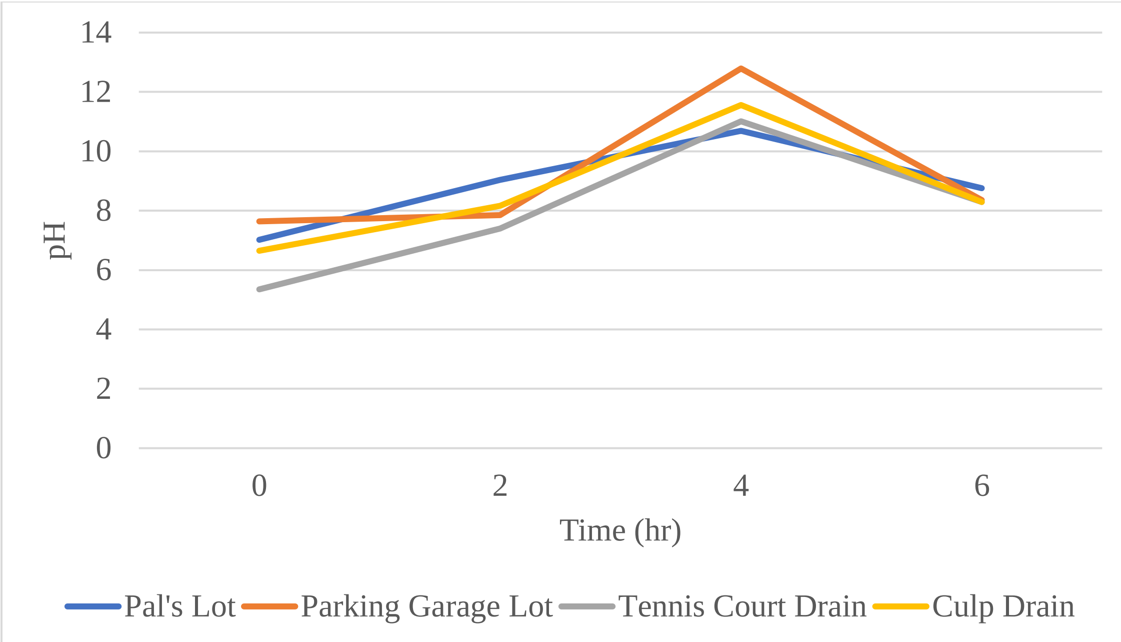

Figure 5. Single pH measurements collected every two hours over a six-hour rain event on 11/11/2023.

Figure 5 suggests that throughout the course of the recorded rain event, pH values varied at each of the sampling sites, however, there is a notable trend. The Pal's Lot, Tennis Court Drain, and Culp Drain all had steady increases in pH over the first four hours of the event. The Parking Garage Lot site remained at a consistent pH until hour 2, between hours 2 and 4 pH increased dramatically. Strikingly, all the sites' pH level peaked at hour 4 and decreased rapidly until the last samples at hour 6.

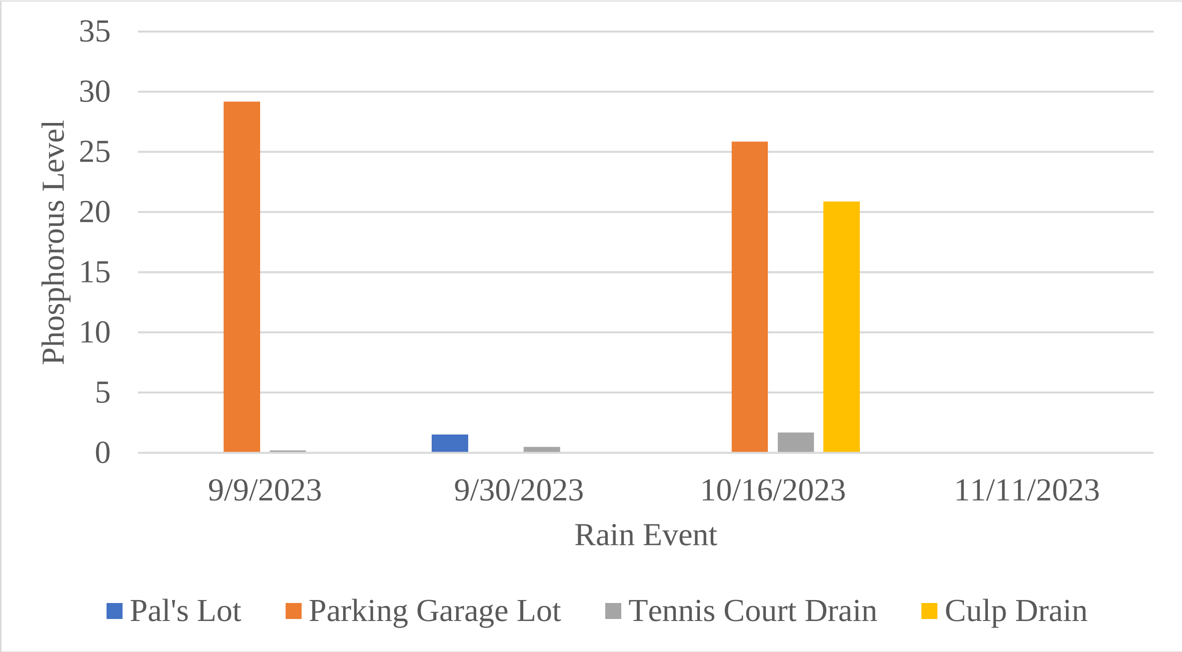

Figure 6. Individual phosphorous measurements at each collection site during the four rain events sampled.

Samples collected at each site during the four recorded rain events rarely contained phosphorous at detectable levels (>0.01) which did not allow for trends to occur which is demonstrated in Figure 6. Samples from the first rain event on 9/9/2023 detected phosphorous at levels of 29.2 at the Parking Garage Lot and 0.19 at the Tennis Court Drain. On 9/30/2023, phosphorous was detected at the Pal's Lot at a level of 1.50 and at the Tennis Court Drain at a level of 0.05. The third rain event, 10/16/2023, had phosphorous at three of the sites, the Parking Garage Lot, the Tennis Court Drain, and the Culp Drain at levels of 25.9, 1.69, and 20.9 respectively. No phosphorus was detected at any site during the 11/11/2023 event. Nitrate was also tested and was only detected at a level of 0.05 at the Culp Drain site on the 9/30/2023 rain event.

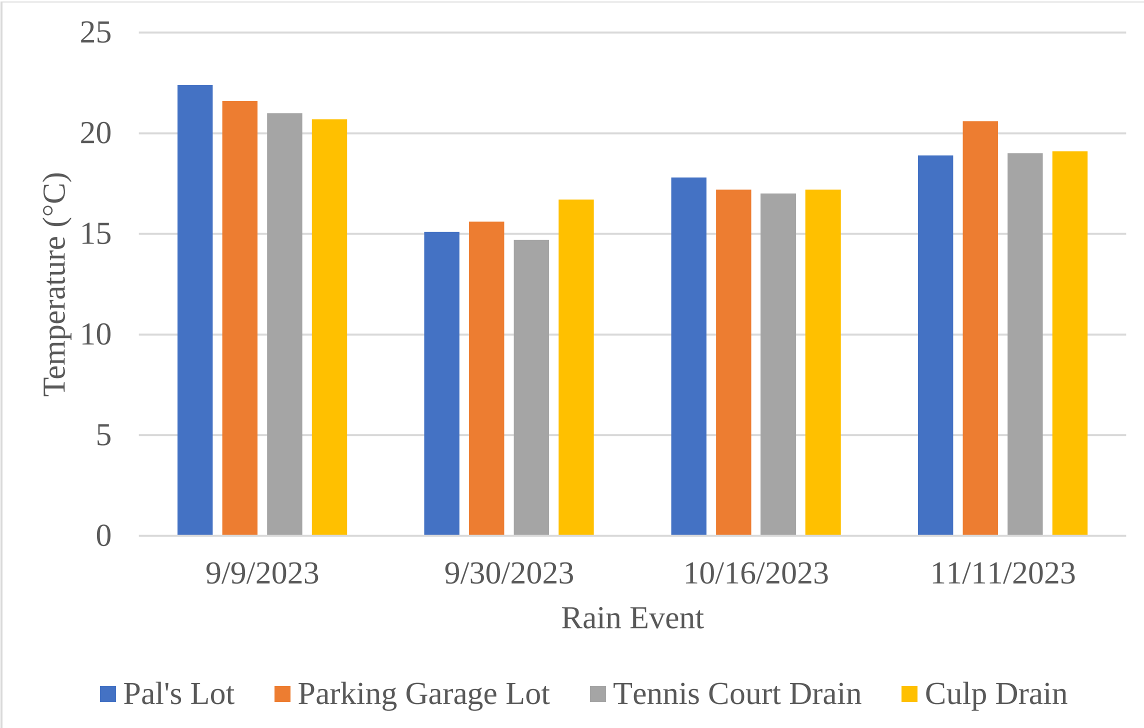

Figure 7. Individual temperature measurements at each collection site during the four rain events sampled.

Although the variation of temperature is not identical across the collection sites, the trend amongst the rain events is prominent. The Pal's Lot had the warmest of its measurements on 9/9/2023 at 22.4°C which decreased to 15.1°C during the 9/30/2023 event which was the lowest Pal's Lot measurement. The following two rain events had a constant increase in temperature at the Pal's Lot. Similarly, the Parking Garage Lot recorded the warmest temperature on 9/9/2023 at 21.6°C which decreased to the coldest measurement at 15.6°C during the 9/30/2023 event. The temperature at the Parking Garage Lot site rose steadily during the other two rain events. Once again, the Tennis Court Drain measured the warmest on 9/9/2023 at 21.0°C, dropped to the coldest measurement at 14.7°C on 9/30/2023, then had increases in temperature over the next two events. The Culp Drain exhibited a similar trend to the other sites, on 9/9/2023 the highest temperature was recorded at 20.7°C, the second event 9/30/2023 had the lowest recording at

16.7°C, the temperature then steadily increased. Figure 7 demonstrates the decreasing then consistent increasing trend of temperature amongst the individual rain events.

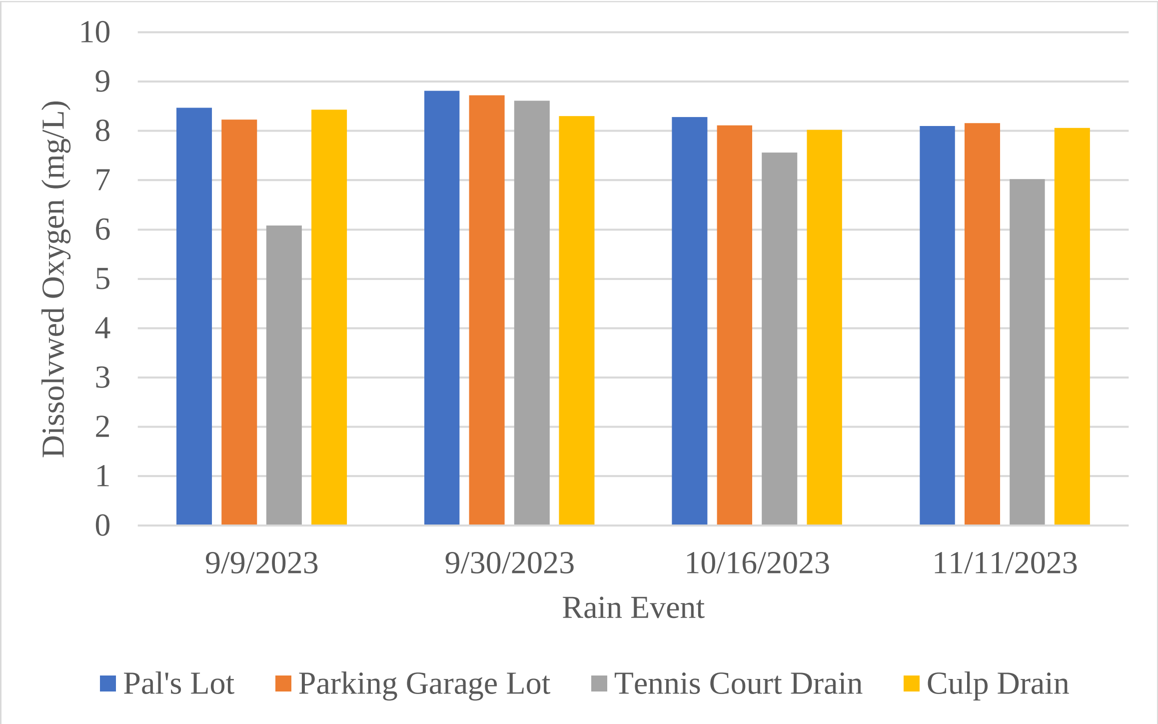

Figure 8. Individual dissolved oxygen measurements at each collection site during the four rain events sampled.

It is demonstrated that dissolved oxygen levels across the 4 recorded rain events at each site were consistent throughout the collection sites (Fig. 8). The Pal’s Lot site had persistent measurements of around 8.0 mg/L with readings between 8.1 mg/L and 8.8 mg/L throughout the individual rain events. Similar to the Pal’s Lot readings, the Parking Garage Lot remained around 8.0 mg/L with readings between 8.1 mg/L and 8.7 mg/L. The Tennis Court Drain site had some variation amongst the collection sites with measurements ranging from 6.1 mg/L to 8.6 mg/L. Comparable to the first two sites, the Culp Drain collection site had readings around 8.0 mg/L ranging from 8.0 mg/L to 8.4 mg/L. Although there are small fluctuations in the Pal’s Lot, Parking Garage Lot, and Culp Drain, all the sample measurements are between 8.0 and 9.0 mg/L.

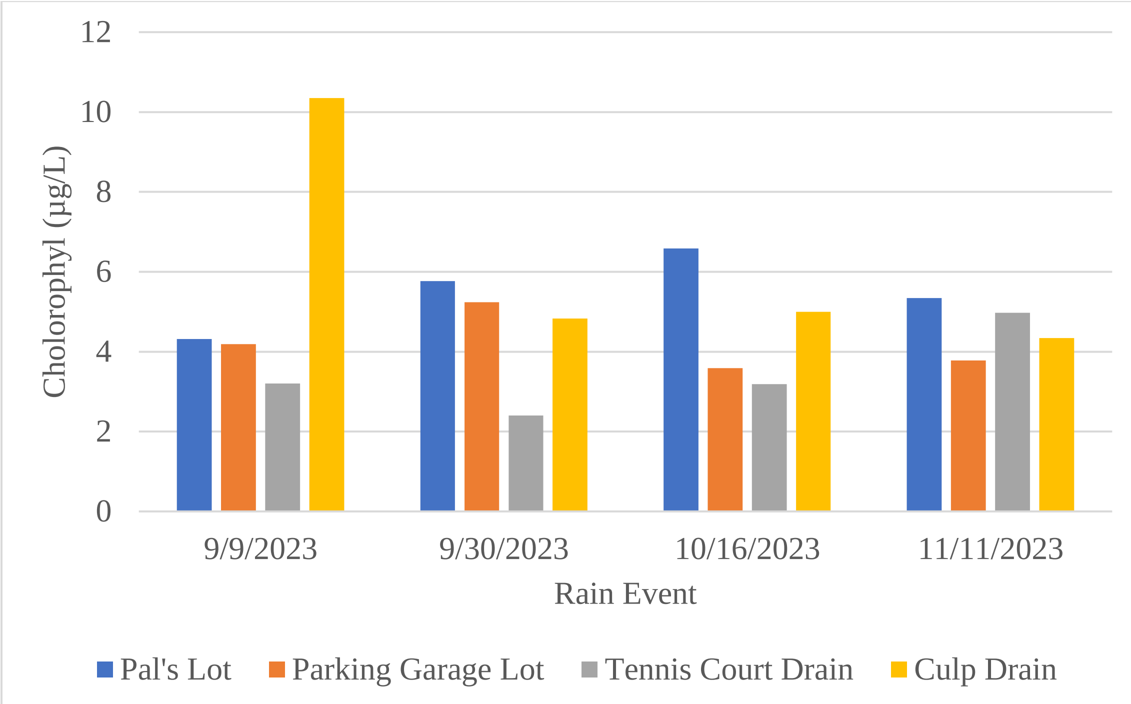

Figure 9. Individual chlorophyll measurements at each collection site during the four rain events sampled.

Chlorophyll levels throughout sampling during the four rain events had variation amongst the collection sites without an apparent trend as seen in Figure 9. The Pal's Lot experienced the highest reading on 10/16/2023 at a level of 6.58 µg/L and the lowest reading on 9/9/2023 at 4.32 µg/L. During the 9/30/2023 rain event the Parking Garage Lot had the highest level recorded at 5.24 µg/L but the lowest level of 3.59 µg/L during the next event on 10/16/2023. The Tennis Court Drain had the highest recording of 4.97 µg/L on 11/11/2023 and the lowest recording overall at 2.40 µg/L on 9/30/2023. During the first rain event, 9/9/2023, the Culp Drain had a high chlorophyll level at 10.4 µg/L which was the highest overall. On the other hand, the Culp Drain experienced its lowest reading on 11/11/2023 at 4.34 µg/L.

Appendix A

Parameter	Pal's Lot	Parking Garage Lot	Tennis Court Drain	Culp Drain
Temp (˚C) Mean	18.8	21.6	17.9	18.4
Temp (˚C) Standard Error	1.51	1.41	1.35	0.92
Temp (˚C) Range	15.1-22.4	15.6-21.6	14.7-21	16.7-20.7
mmHg Mean	714	718.4	714.3	713.7
mmHg Standard Error	706.3-719.8	705.3-718.4	705.5-720	704.9-720.5
mmHg Range	2.91	2.86	3.09	3.24
DO (mg/L) Mean	8.31	8.23	7.32	8.2
DO (mg/L) Standard Error	0.58	0.11	0.15	0.27
DO (mg/L) Range	6.08-8.47	8.3-8.81	7.56-8.28	7.02-8.16
SPC (µS/cm) Mean	72.5	57	98.3	70.6
SPC (µS/cm) Standard Error	13.95	10.66	8.8	10.45
SPC (µS/cm) Range	41.4-104.4	34-84.6	79.9-119	50.4-93.2
SAL-ppt Mean	0.04	0.02	0.05	0.03

SAL-ppt Standard Error	0.009	0.006	0.004	0.009
SAL-ppt Range	0.02-0.06	0.02-0.04	0.03-0.05	0.01-0.05
pH Mean	8.38	8.1	7.31	7.77
pH Standard Error	0.46	0.16	0.65	0.37
pH Range	7.02-9.05	7.64-8.23	5.35-8.06	6.65-8.19
Chl (µg/L) Mean	5.5	4.2	3.44	6.13
Chl (µg/L) Standard Error	1.63	0.75	0.77	0.34
Chl (µg/L) Range	3.2-10.35	2.4-5.77	3.19-6.58	3.78-5.34

Table 1. Mean, standard error, and range for each parameter measured throughout the rain events at each of the collection sites.

CHAPTER 4: DISCUSSION

Stormwater Toxicity to D. magna

This study found little to no mortality of *D. magna* after acute exposure to stormwater runoff samples collected at the beginning and end of a rain event. The only recorded mortalities were observed in organisms exposed to samples collected from the Culp Drain, Pal's Lot, and Parking Garage Lot site at the beginning of the rain event. Similarly, McQueen et al. (2010) investigated polluted runoff effects on *C. dubia* and *P. Promelas.* They found that mortality (20%) in *C. dubia* was limited to those exposed to the very first sample taken during the rain event. These findings are likely the result of a "first flush effect" which is the high initial concentration of pollutants found in runoff at the beginning of a rain event. Similarly, the present study used samples from the first flush of a rain event to determine toxicity using *D. magna.* Kim et al. (2005) found that there are three categories of first flush, high first flush where 50% of contaminants are washed off in the first 30% of the rain event, medium first flush where 30-50% of contaminants are washed off in the first 30% of the rain event, and non-first flush where less than 30% contaminants are washed off in the first 30% of the rain event (Kim et al., 2005). The pollutants found in a first flush event include harmful nitrates and phosphates, heavy metals, and oils (Han et al., 2006; Lee et al., 2002). These contaminants being deposited at high concentrations into the nearby ecosystems are causing harm to the organisms as well.

The *D. magna* data contradicted some recent studies. Wedel and Jackson (2023) found that *D. magna* had irreversible morphological damage when exposed to urbanized and industrial stormwater runoff. After exposing *D. magna* to different sample sites such as parks, creeks, rivers, and drainpipes, it was found that the drainpipe caused 100% mortality while the creek sample was the least lethal with only 20% mortality. Samples from every site did cause *D.*

magna mortality as well as morphological damage (Wedel & Jackson, 2023). Marsalek et al. (1999) found that exposure to highway 22% severe toxicity levels where runoff samples from a busy two-lane divided highway were taken. The toxicity level was determined by a 75% mortality rate in *D. magna* with the surviving organisms having less than 9% activity after 48 hours. Their findings suggest that runoff from impervious surfaces such as highways can be lethal to invertebrates (Marsalek et al., 1999). These studies were focused in large cities that produce more pollution overall which can be picked up by runoff and can cause higher amounts of damage to nearby ecosystems. ETSU is a smaller campus with a moderate amount of traffic which may not cause an adequate accumulation of pollutants that can cause harm to organisms. Future studies should examine land uses that have potential for higher concentrations of pollutants in stormwater runoff.

Stormwater Chemical Parameters

Differences in the water quality parameters recorded during the four rain events at different collection sites were not statistically significant. Specific conductivity, pH, phosphate, nitrate, and chlorophyll$_a$ levels were variable over the four rain events at the collection sites with no discernable trends. Dissolved oxygen and pH remained consistent throughout the sampling. The time course rain event did show interesting trends with the pH and specific conductivity parameters, pH had a peak at hour four of testing and specific conductivity had a spiked increase at hour four.

Butler and Vasconcelos (2015) performed an 18-month study monitoring highway stormwater runoff pH, temperature, and conductivity affecting a creek. They found that there was a spike in conductivity immediately after rain events whereas pH and temperature remained consistent throughout. Due to the consistency of the parameters these findings suggested that the runoff did not have effects on the creek (Butler & Vasconcelos, 2015). Similarly, the present study found the pH and temperature to be consistent throughout individual rain events. Throughout the time course of this study, a spike in conductivity was seen in hour 6 contradicting Butler and Vasconcelos' finding that conductivity spiked at the beginning of the event. This contradiction could be because of differences in land use, the present study focused on roads and drains with impervious surfaces while Butler and Vasconcelos focused on agricultural areas. Since conductivity is influenced by inorganic dissolved solids such as chloride, sulfate and iron which can all be found in agricultural landscapes (EPA 2012). Izonfuo and Bariweni (2010) conducted a study to determine the effect of runoff on pollution of a creek. Nitrates and phosphates were both tested at sample sites down the creek and found to have significantly higher concentrations at the downstream site. This suggests that the runoff picked up more phosphates and nitrates and carried them downstream. Similar results were found in the same study when investigating specific conductivity and pH. Specific conductivity and pH both had significant increases in the downstream site (Izonfuo & Bariweni, 2010). This study found that nitrate and phosphorous did not increase as a rain event continued and were only found sporadically which is contradictory of Izonfuo and Bariweni. However, the pH and specific conductivity did show an increase throughout the time course rain event which is supportive of Izonfuo and Bariweni's data. Once again, Izonfuo and Bariweni focused on agricultural land uses whereas the present study focused on impervious roads and drains which could be cause for the contradiction. A study done by the faculty and students of the Geosciences Department at ETSU investigated pH, conductivity, and dissolved oxygen (DO) on campus (Luffman, personal communication, August 30, 2022). They found as an individual rain event continued, the conductivity and DO decreased while the pH increased. The present study also found an increase in pH up to hour 6 and a constant increase in specific conductivity over a rain event. DO was not measured throughout the time course rain event but was consistent throughout individual rain events.

Differences in land use have been shown to alter the composition of stormwater runoff. Urban areas tend to have more pollution which creates a higher concentration of contaminants in surface runoff than in rural areas (Sajikumar & Remya, 2015). Since urban areas have so many impervious surfaces, vehicular traffic, and higher human population, toxicants such as metals, hydrocarbons, and pesticides accumulate (Brown et al., 2012). Rural areas still have potential to introduce contaminants through agriculture, livestock, and pesticide use however, there is less accumulation of pollutants than urban areas (Tran et al., 2019). Pollutants such as oils, grease, fuels, heavy metals, and road salts have been found in highway runoff because of the impervious nature of the asphalt which can cause damage to the surrounding ecosystems as well (Kim et al., 2005). In the present study, the Pal's Lot and Parking Garage Lots were selected to be representative of a place with high vehicular traffic and one with low vehicular traffic while the Culp Drain and Tennis Court Drain were picked because of a high potential of contamination based off of a study done by the ETSU Geology Department. Land use differences were not reflected in the toxicity or water chemistry data which is contradictory of previous studies (Luffman, personal communication, August 30, 2022). It is possible that these sites had human populations and vehicular traffic levels too similar to one another to see any differences in the water chemistry. Also, all the sites were located on impervious surfaces which allow pollutants to accumulate easier.

Summary

This study tested water chemistry parameters pH, temperature, dissolved oxygen, specific conductivity, phosphorous, nitrate, and chlorophyll of stormwater runoff at different locations on the ETSU campus. Sampling sites were based off the varying possibilities of contaminants at each location. The stormwater parameters showed no significant difference amongst the different collection sites, suggesting stormwater parameters are not different across locations on campus. Since this study was comparative and only observing the contamination of the sites there was no need for a stormwater control . Acute toxicity 48-h tests were also performed exposing *D. magna* to samples from each site at the beginning and end of a rain event. *D. magna* did not experience high levels of mortality after exposure to any of the samples indicating the runoff collected was not toxic to invertebrates. As such, the data that were collected do not support the predictions that stormwater from campus collection sites with the highest amount of vehicular traffic would be more toxic to *D. magna* as indicated by 48-h acute toxicity tests. It also did not support the prediction that values for a suite of basic water chemistry parameters collected from the sites would differ between those with greater vehicular traffic and those without. Although no significant toxicity was found, these conclusions may be indicative of good receiving systems that collect runoff on the ETSU campus.

REFERENCES

American Society for Testing and Materials. (1991). *Standard guide for conducting the frog embryo teratogenesis assay-xenopus (FETAX)*.

Bosisio, S., Fortaner, S., Bellinetto, S., Farina, M., Del Torchio, R., Prati, M., Gornati, R., Bernardini, G., & Sabbioni, E. (2009). Developmental toxicity, uptake and distribution of sodium chromate assayed by frog embryo teratogenesis assay-xenopus (FETAX). *Science of The Total Environment*, *407*(18), 5039–5045. https://doi.org/10.1016/j.scitotenv.2009.05.047

Brown, M. G., Dobbs, E. K., Snodgrass, J. W., & Ownby, D. R. (2012). Ameliorative effects of sodium chloride on acute copper toxicity among Cope's gray tree frog (Hyla Chrysoscelis) and green frog (Rana Clamitans) embryos. *Environmental Toxicology and Chemistry*, *31*(4), 836–842. https://doi.org/10.1002/etc.1751

Burant, A., Selbig, W., Furlong, E. T., & Higgins, C. P. (2018). Trace organic contaminants in urban runoff: Associations with urban land-use. *Environmental Pollution*, *242*, 2068–2077. https://doi.org/10.1016/j.envpol.2018.06.066

Butler, C. G., & Vasconcelos, J. G. (2015). The effect of highway stormwater runoff on water quality in the little cahaba creek. *World Environmental and Water Resources Congress 2015*. https://doi.org/10.1061/9780784479162.261

Environmental Protection Agency. (2012). *5.9 conductivity*. EPA. Retrieved from https://archive.epa.gov/water/archive/web/html/vms59.html#:~:text=Conductivity%20in%20water%20is%20affected,that%20carry%20a%20positive%20charge).

Gardner, K. M., & Royer, T. V. (2010). Effect of road salt application on seasonal chloride concentrations and toxicity in south-central Indiana Streams. *Journal of Environmental Quality*, *39*(3), 1036–1042. https://doi.org/10.2134/jeq2009.0402

Haake, D. M., Krchma, S., Meyners, C. W., & Virag, R. (2022). Impacts of urbanization on chloride and stream invertebrates: A 10-year citizen science field study of road salt in stormwater runoff. *Integrated Environmental Assessment and Management*, *18*(6), 1667–1677. https://doi.org/10.1002/ieam.4594

Han, Y., Lau, S.-L., Kayhanian, M., & Stenstrom, M. K. (2006). Characteristics of highway stormwater runoff. *Water Environment Research*, *78*(12), 2377–2388. https://doi.org/10.2175/106143006x95447

Izonfuo, L. W. A., & Bariweni, A. P. (2010). The effect of urban runoff water and human activities on some physico- chemical parameters of the Epie Creek in the Niger Delta. *Journal of Applied Sciences and Environmental Management*, *5*(1). https://doi.org/10.4314/jasem.v5i1.54941

Kim, L.-H., Kayhanian, M., Zoh, K.-D., & Stenstrom, M. K. (2005). Modeling of highway stormwater runoff. *Science of The Total Environment*, *348*(1-3), 1–18. https://doi.org/10.1016/j.scitotenv.2004.12.063

Lee, J. H., Bang, K. W., Ketchum, L. H., Choe, J. S., & Yu, M. J. (2002). First flush analysis of urban storm runoff. *Science of The Total Environment*, *293*(1-3), 163–175. https://doi.org/10.1016/s0048-9697(02)00006-2

Luffman, I. personal communication, August 30, 2022

Marsalek, J., Rochfort, Q., Brownlee, B., Mayer, T., & Servos, M. (1999). An exploratory study of urban runoff toxicity. *Water Science and Technology*, *39*(12), 33–39. https://doi.org/10.2166/wst.1999.0526

McQueen, A. D., Johnson, B. M., Rodgers, J. H., & English, W. R. (2010). Campus parking lot stormwater runoff: Physicochemical analyses and toxicity tests using Ceriodaphnia dubia and Pimephales Promelas. *Chemosphere*, *79*(5), 561–569. https://doi.org/10.1016/j.chemosphere.2010.02.004

Post, A. K., & Knapp, A. K. (2020). Review for "The importance of extreme rainfall events and their timing in a semi-arid grassland". *Journal of Ecology*, *108*(6), 2431–2443. https://doi.org/10.1111/1365-2745.13478/v1/review2

Sajikumar, N., & Remya, R. S. (2015). Impact of land cover and land use change on runoff characteristics. *Journal of Environmental Management*, *161*, 460–468. https://doi.org/10.1016/j.jenvman.2014.12.041

Sievers, M., Hale, R., Swearer, S. E., & Parris, K. M. (2018). Contaminant mixtures interact to impair predator-avoidance behaviours and survival in a larval amphibian. *Ecotoxicology and Environmental Safety*, *161*, 482–488. https://doi.org/10.1016/j.ecoenv.2018.06.028

Tran, N. H., Reinhard, M., Khan, E., Chen, H., Nguyen, V. T., Li, Y., Goh, S. G., Nguyen, Q. B., Saeidi, N., & Gin, K. Y.-H. (2019). Emerging contaminants in wastewater, stormwater runoff, and surface water: Application as chemical markers for diffuse sources. *Science of The Total Environment*, *676*, 252–267. https://doi.org/10.1016/j.scitotenv.2019.04.160

United States Environmental Protection Agency, Office of Water. (2002). *Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms* (5th ed.).

Wedel, L., & Jackson, L. (2023). Toxicity effects in daphnia magna from seasonal stormwater runoff. *SSRN Electronic Journal*. https://doi.org/10.2139/ssrn.4360533

Young, A., Kochenkov, V., McIntyre, J. K., Stark, J. D., & Coffin, A. B. (2018). Urban stormwater runoff negatively impacts lateral line development in larval zebrafish and salmon embryos. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-21209-z