Water Quality and Nutrient Fluxes in Tributaries of the Western **Branch of the Susquehanna River**

Introduction

- The Susquehanna River receives nutrients and sediment from a variety of sources which it then delivers to the Chesapeake Bay (Zhang *et al.* 2013). These nutrients, typically nitrate, ammonium, and phosphate, cause water health to decrease through eutrophication, causing dead zones (Zhang et al. 2013). Major sources of nutrient pollution include agricultural practices (fertilizing and tilling), improperly drained storm water, and various industrial practices (Zhang et al. 2013). Pollution of the Chesapeake Bay has decreased in recent decades (Landers 2010). Between 1985 and 1998, there was a 12-25% decrease in nitrogen levels and 36-60% decrease in phosphorus levels (Belval and Sprague 1999). The implementation of best management practices (BMPs), practices that dictate appropriate engineering and agricultural standards and are meant to reduce runoff of nutrient-rich sediment, and the ban on phosphate detergents that occurred in 1989 may have contributed to this decrease (Belval and Sprague 1999; Crable 2010). Despite this, high nitrogen and phosphorus levels still cause algal blooms and dead zones in the Chesapeake Bay, creating a need for further research (Zhang 2013). Previous studies tested tributaries in the area of the Conowingo Reservoir in Maryland, but tributaries in the western branch of the Susquehanna River are unexplored (Zhang *et al.* 2013).
- The purpose of this experiment is to quantify the amounts of ammonium, phosphate, and nitrate contributed over time to the western branch of the Susquehanna River by nine tributaries. This study attempts to pinpoint major contributors to the pollution of the Susquehanna River's western branch. It also examines the impact of land use on pollution by sorting the nine tributaries into three categories: agricultural, forested, and urban (Figure 1.).
- It is hypothesized that there will be a statistical difference between tributaries in areas that are highly agricultural and tributaries in urban or forested areas in the amount of dissolved inorganic nitrogen (DIN; includes ammonium and nitrate) and phosphate contributed to the Susquehanna River due to the use of nutrient-rich fertilizers in these areas.

Materials and Methods

Collecting Water Samples

- Water samples were collected from 5/21/15 to 8/23/15 bi-weekly at nine tributaries
- Used a syringe with a microfiber glass filter to transfer water into acid-washed bottles
- Samples were frozen until nutrient testing was performed
- pH, conductivity, dissolved oxygen, temperature, flow, turbidity, and depth were found at each site at the time samples were collected

Spectrophotometry

- Nitrate, ammonium, and phosphate levels of water samples were found using spectrophotometry
- In a 96-well plate, water samples and various reagents were combined to create a color reaction
- Absorbance levels were read and compared to standard values
- Used two one-way ANOVA tests to compare phosphate levels and dissolved inorganic nitrogen levels of the tributaries (Figure 2.).

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A Forested Urban Agricultural Tunkhannock Creek Whitlock Creek Sutton Creek 💮 Hunlock Creek Shickshinny Creek Gardner Creel Harvey's Creek Google earth 10.68 mi Figure 1. Map of the nine tributaries that were sampled. Forested tributaries included Shickshinny Creek, Hunlock Creek, and Harvey's Creek. Urban tributaries included Mill Creek and the Lackawanna River. Agricultural tributaries included Tunkhannock Creek, Sutton Creek, Whitlock Creek, and Gardner Creek. DIN Flux (kg/day) 2.5 Figure 2. A. Box plot **For Tributaries Over Time** showing flux rates of dissolved inorganic $\widehat{\geq}$ 2.0 nitrogen (DIN) in and (kg pe the statistical similarities between **A**. the tributaries. The 1.0 letters above the boxes signify 0.5 statistically comparable groups 0.0 (p > 0.05). All of the 0.05 Phosphate Flux (kg/day) for Tributaries agricultural tributaries, except b.c.d a.b.c.d Gardner Creek, are in a statistically exclusive group for DIN flux. **B**. 0.03 **B**. Box plot showing flux rates of phosphate **_**____ 0.02 · (PO_4) in and the statistical similarities between the tributaries. The letters 0.00 above the boxes signify statistically comparable groups (p > 0.05). References Belval, D., & Sprague, L. (1999). Monitoring Nutrients in the Major Rivers Draining to Chesapeake Bay. U.S. Geological Survey and Water-Resources Investigations Report. Retrieved November 24, 2015. Crable, A. (2010, July 6). Phosphate ban in dishwasher detergent goes into effect. Retrieved December 1, 2015. Horwarth, R., Swaney, D., Boyer, E., Marino, R., Jaworski, N., & Goodale, C. (2006). The influence of climate on average nitrogen export from large watersheds in the Northeastern United States Landers, J. (2010). Federal Agencies Release Plan For Improving Health of Chesapeake Bay. *Civil Engineering*, 80(7), 28-30. Retrieved November 24, 2015.

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Figure 3. Graphs showing the amount of DIN (kg) flowing through each tributary per day over the sampling period. As shown by **Figure 2A.** and the above, Mill Creek showed the greatest change in DIN compared to all other tributaries.



Figure 4. Graphs showing the amount of phosphate (kg) flowing through each tributary per day over the sampling period. As shown by **Figure 2A.** and the above, Sutton Creek showed the most change in DIN compared to all other tributaries.

Conclusions

- There were statistical differences between tributaries for DIN (Figure 2A.).
- Most notably, three of four agricultural tributaries were statistically different from tributaries in urban and forested areas. This could be a result of agricultural practices, such as runoff of fertilizers (Zhang *et al.* 2013).
- Though most of the mean DIN flux rates were less than 1 kg/day, the total amount 3960 kg of DIN the Susquehanna receives annually (Horwarth et al. 2006).
- There were statistical differences between tributaries for phosphate, but there was no correlation between phosphate flux rates and land use (Figure 2B.).
- Overgrowth prevented access to three tributaries (Lackawanna River, Shickshinny Creek, and Whitlock Creek) from July 1st on.
- Further research would examine land use more thoroughly through remote sensing. Additionally, more tributaries should be examined to understand the contribution of the western branch to the pollution of the Chesapeake Bay. Finally, the project should be extended for a longer period of time to understand seasonal trends in nutrient levels and fluxes. This would allow identification of the biggest contributors of nutrient pollution to the Susquehanna River so preventative actions can be taken.



Water Quality and Nutrient Fluxes in Tributaries of the Northern Area of the Main Stem of the Susquehanna River

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Introduction

The Susquehanna River receives nutrients – most notably nitrate, ammonium, and phosphate – from a variety of sources (Zhang et al. 2013). These nutrients originate from sources such as: livestock wastewater, runoff of crop fertilizers, urban runoff and sewage effluents, and waste from industrial sites (Camargo and Alonso 2006; Zhang et al. 2013). These sources can either be point or non-point sources. Point sources are sources of nutrients that can be attributed to a certain location. Non-point sources of nutrients are from a large area and can be from a variety of sources (Belval and Sprague 1999). It then delivers these nutrients to the Chesapeake Bay.

Increased dissolved nutrient levels and sediment inputs have resulted in phytoplankton blooms, low water clarity, and low dissolved oxygen (Zhang et al. 2013). Phytoplankton bloom, die, and are decomposed by bacteria. The bacteria convert the nutrients in the phytoplankton to inorganic compounds (including nitrate, ammonium, and phosphate), consuming dissolved oxygen in the process (Nixon 1995). The low dissolved oxygen levels can cause areas with unsuitable conditions for other species to survive, called "dead zones" (Chorus and Bartram 1999). When this occurs, it can affect the health of the Chesapeake's delicate ecosystem (Zhang et al. 2013).

Increased nitrate levels are also very unsafe for humans. When in drinking water, nitrate can cause a disease that causes hemoglobin to oxidize to methemoglobin. This makes oxygen incapable of binding, leading to low oxygen saturation in the blood of animals (Kumar and Puri

2012). Additionally, fish that live in environments with nitrate concentrations of 5-6 mg/L may not grow properly (Camargo et al. 2004). Fish that have been raised in high-nitrogen environments can pass the nitrates on to humans when consumed. This can cause various digestive cancers in humans (Camargo and Alonso 2006). However, ammonium levels would have to be very high (75-360 mg/kg body weight) or higher for short-term negative effects, and even higher (200-500 mg/kg body weight) to produce negative effects in most animals (World Health Organization 1996). Additionally, phosphate levels would have to be present at very high levels in order to be harmful to humans or animals (not counting the damaging effects of eutrophication) (Kumar and Puri 2012).

Previous decades have shown a downward trend in nutrient levels of the Susquehanna River. Nitrogen levels decreased between 12 and 25% between 1985 and 1998. This could be due to best management practices (BMPs). BMPs dictate appropriate engineering and agricultural standards that are meant to reduce nutrient runoff and sediment. Additionally, phosphorus levels decreased between 36 to 60% during 1985 and 1998. During this period, a ban was placed on phosphate detergents, and additional agricultural BMPs were implemented (Belval and Sprague 1999). However, the water quality of the Chesapeake Bay has been decreasing in recent decades (Landers 2010). Since the Susquehanna River is the main body of water flowing into the Chesapeake, it is very important to understand the effects of the Susquehanna River on the Chesapeake's water quality. It is estimated that nitrogen fluxes in the Susquehanna River to the Chesapeake Bay can increase 3-17% by 2030, and 16-65% by 2095 if action is not taken (Howarth et al. 2006). This increase is expected to occur because of an increase in human activities that produce nitrogen pollution including the use of fertilizers, storm water drainage, and waste water drainage (Howarth et al. 2006). Previous studies regarding water quality and nutrient fluxes have been conducted in the Conowingo Reservoir area and the Lower Susquehanna River Reservoir System, but no studies have been conducted in the northern area of the Susquehanna River (Zhang et al. 2013). Since it is estimated that nutrient levels will increase, it is imperative to discover where nutrients are originating. Additionally, since many people fish in creeks around the northern area of the Susquehanna, it is important to make sure the fish are safe for consumption (Camargo and Alonso 2006).

The purpose of this experiment is to pinpoint major contributors to pollution of the Susquehanna River by quantifying the amounts of ammonium, phosphate, and nitrate contributed over time by nine tributaries. It also examines the impact of land use on pollution by sorting the nine tributaries into three categories: agricultural, forested, and urban (Figure 1). It is hypothesized that there will be a statistical difference between tributaries in areas that are in highly agricultural areas and tributaries in urban or forested areas in the amount of dissolved inorganic nitrogen (DIN; includes ammonium and nitrate) and phosphate contributed to the Susquehanna River. This will be due to practices used in farming such as fertilizing and waste from livestock (Zhang et al. 2013). Additionally, tributaries in forested areas will remain mostly consistent in nutrient values since there are fewer sources of nutrients (Camargo and Alonso 2006). In terms of tributaries in urban areas, results are not expected as it depends heavily on the effectiveness of the city's waste and storm water removal (Camargo and Alonso 2006).

Methods

Sampling Sites

Nine tributaries of the Susquehanna River were selected for testing: Shickshinny Creek (41° 9'5.74"N, 76° 8'51.53"W), Hunlock Creek (41°12'38.21"N, 76° 5'21.12"W), Harvey's

Creek (41°14'5.48"N, 76° 0'6.22"W), Sutton Creek (41°23'13.84"N, 75°50'48.33"W), the Lackawanna River (41°21'17.67"N, 75°45'48.88"W), Whitlock Creek (41°26'25.33"N, 75°51'27.35"W), Mill Creek (41°15'25.64"N, 75°50'53.74"W), and Gardner Creek (41°23'35.60"N, 75°49'3.44"W). These sites were selected to sample a variety of land uses and for accessibility close to the mouth of the tributary. For aerial views and on-site pictures, see Appendix 1.



Figure 1. Map of the nine tributaries that were sampled. Forested tributaries included Shickshinny Creek, Hunlock Creek, and Harvey's Creek. Urban tributaries included Mill Creek and the Lackawanna River. Agricultural tributaries included Tunkhannock Creek, Sutton Creek, Whitlock Creek, and Gardner Creek.

The tributaries were sampled bi-weekly from May 21st, 2015 to August 23rd, 2015. For each tributary, readings were taken in triplicate, approximately one meter apart, at each tributary. These readings were taken as close to the mouth of the tributary as could be accessed. pH, temperature, dissolved oxygen and conductivity were measured in situ. One measurement for depth was found for each tributary at the deepest part of the area of sampling. Flow was also found at this point. A water sample was taken from each tributary and tested for turbidity.

Three water samples were collected using a syringe equipped with a glass microfiber filter (GFF) to ensure only dissolved nutrients were collected. Water was filtered into acidwashed polypropolene bottles. The samples were kept on ice until the water could be frozen to await nutrient testing.

Nutrient Testing (using EPA-approved Methods):

Testing for Nitrate

Standards were created from 100 ppm to 0.01 ppm using 1000 ppm nitrate. The first reagent was created by dissolving 0.5 g sulfanilamide in no more than 30 mL of distilled water. This solution was poured into a 50 mL volumetric flask, and 15 mL of concentrated HCl were added. Distilled water was added until the solution was 50 mL. The second reagent was created by dissolving 0.076 g NED in 50 mL of distilled water. An assay buffer was prepared by dissolving 0.19 g potassium phosphate monobasic, 0.070 g potassium hydroxide, and 0.465 EDTA in 50 mL of distilled water.

Standards from 0.01 to 10 were added in duplicate to a clean, disposable, and clear polystyrene flat-bottom 96-well plate. 100 μ L of sample were added to an individual well. Each plate was created in duplicate. An enzyme solution was then created. A vial containing NaR was removed from a foil pouch and the tube was tapped to settle the contents. The contents of an enzyme diluent squeeze bulb were completely emptied into the NaR vial. The cap was replaced and mixed by inversion three times. The vial was allowed to stand at room temperature for ten minutes, mixing at five and ten minutes, and kept on ice during use. To create an NADH solution, the tube was removed and tapped to settle the contents. 1.5 mL of distilled water was

added to the tube. The tube was then mixed by inversion and kept on ice during use. To create the enzyme solution, 9 mL assay buffer, 1 mL NaR solution, and 0.5 mL NADH solution were added to a pipette reservoir basin and mixed thoroughly.

 $50 \ \mu\text{L}$ of mixed enzyme solution were added to each well of the plates, and vortexed at 900 rpm for 60 minutes. $50 \ \mu\text{L}$ of the first reagent and the second reagent were added to each well. The plates were vortexed for ten minutes at 600 rpm. Absorbencies were found at 540 nm (Mischler 2015).

Testing for Orthophosphate

Standards were created from 100 ppm to 0.01 ppm from 1000 ppm orthophosphate. The first reagent was created by adding 8.6 mL of concentrated sulfuric acid to 30 mL of distilled water in a 50 mL volumetric flask. Once the solution cooled, 0.877 g ammonium molybdate was added. Distilled water was added until the solution was 50 mL. To prepare the second reagent, 0.175 g of PVA was added to a beaker containing 40 mL of 80°C distilled water. Once the solution cooled to room temperature, 0.0175 of malachite green carbinol hydrochloride was added. Distilled water was added until the volume of the solution was 50 mL.

Standards from 0.01 to 10 were added in duplicate to a clean, disposable, and clear polystyrene flat-bottom 96-well plate. 200 μ L of sample were added to an individual well. Each plate was created in duplicate. 40 μ L of the first reagent were added to each well and vortexed at 400 rpm for ten minutes. 40 μ L of the second reagent were added to each well and vortexed at 750 rpm for twenty minutes. Absorbencies were found at 630 nm (Mischler 2015).

Testing for Ammonium

Standards were created from 100 ppm to 0.01 ppm from 1000 ppm ammonium. Three reagents were prepared. The first, 1.632 M trisodium citrate dehydrate (294.10 g/mol) was

prepared by dissolving 24 g trisodium citrate dihyrate in 50 mL of distilled water. To prepare the second reagent, 1.9 g of ground phenol and 0.019 g of sodium nitroprusside were dissolved in 50 mL of distilled water. For the third reagent, 1 g of sodium hydroxide was added to 48 mL of distilled water. 2 mL of ~5% sodium hypochlorite bleach solution were then added.

Standards from 0.01 to 10 were added in duplicate to a clean, disposable, and clear polystyrene flat-bottom 96-well plate. 200 μ L of sample were added to an individual well. Each plate was created in duplicate. 10 μ L of each reagent were added to each well, vortexing at 750 rpm for two minutes after each addition. The plates were then placed in the dark with the lids on for two hours. Absorbencies were found at 630 nm (Mischler 2015).

Nutrient Fluxes

Standard curves were created for each plate using the absorbencies and the ppm of each nutrient. The slope formula for each plate was found, and this was used to find the concentration of each nutrient in each sample. To make nutrient levels more comparable between tributaries and to see the total contribution to the Susquehanna River, nutrient fluxes for DIN and phosphate were calculated by finding the amount of each nutrient that flowed through the tributary in an hour.

Statistical Testing

A one-way ANOVA was created to compare total dissolved inorganic nitrogen (ammonium and nitrate) fluxes between tributaries. Another ANOVA was created to compare phosphate fluxes between tributaries.

Results:

Nutrient Fluxes:



Figure 2. Box plot showing flux rates of dissolved inorganic nitrogen (DIN) and phosphate, and the statistical differences between the tributaries (p=0.05).



Figure 3. Graphs showing the amount of DIN (kg) flowing through each tributary per day over the sampling period. The lines are smoothing lines with a 95% confidence interval.



Figure 4. Graphs showing the amount of dissolved phosphate (kg) flowing through each tributary per day over the sampling period. The lines are smoothing lines with a 95% confidence interval.

Nutrient Concentrations



Figure 5. Graphs showing the concentration of dissolved nitrate values (mg/L). The lines are smoothing lines with a 95% confidence interval.



Figure 6. Graphs showing the concentration of dissolved ammonium values (mg/L). The lines are smoothing lines with a 95% confidence interval.



Figure 7. Graphs showing the concentration of dissolved phosphate values (mg/L). The lines are smoothing lines with a 95% confidence interval. **Discussion:**

As shown in Figure 2, there are statistical differences between tributaries for DIN. Most notably, three of four tributaries in agricultural areas (Sutton Creek, Whitlock Creek, and Tunkhannock Creek) were statistically different from tributaries in urban and forested areas. This could be a result of agricultural practices, such as runoff of fertilizers, animal waste, and tilling of land (Zhang *et al.* 2013). These three tributaries also showed much higher values in DIN than all other tributaries. The Susquehanna River delivers about 3960 kg/year of DIN to the Chesapeake Bay (Horwarth *et al.* 2006). Though most of the mean values for DIN were under 0.5 kg/day, these nine tributaries still contributed a notable amount of DIN to the Susquehanna throughout the sampling period. Mill Creek also showed a lot of variance throughout the year, and showed an upward trend throughout the months. Mill Creek flows directly behind the Mohegan Sun Pocono Arena. Race season starts in April, and continues through the summer until September. The races occur most frequently in August. This could be one reason for the

increase in DIN. It would also make sense that Mill Creek began to show similarities to tributaries in agricultural zones. There were also statistical differences between tributaries for dissolved phosphate, but there were no correlations between phosphate flux rates and land use.

Tunkhannock Creek, Sutton Creek, Whitlock Creek, and later in the season, Mill Creek all showed nitrate concentrations high enough (above 5-6 mg/L) to have harmful effects on fish and humans (Figure 5) (Camargo *et al.* 2004). Though the USA federal government allows drinking water to contain nitrate concentrations up to 10 mg/L, these levels are not enough to prevent fish and other animals from developing incorrectly or becoming sick (Camargo *et al.* 2004). People commonly fish in all three of these creeks, and eating fish caught can lead to digestive cancers and other problems (Camargo and Alonso 2006). Ammonium and phosphate concentrations remained low enough for all tributaries to not be of concern in terms of direct organismal health, but these levels can still cause eutrophication and phytoplankton blooms (Zhang *et al.* 2013).

One problem this study encountered was that three of the nine sampling sites could not be accessed the entire sampling period. These sites included: the Lackawanna River, Shickshinny Creek, and Whitlock Creek. However, since the one-way ANOVA is capable of withstanding slight differences in sample size, it is believed that the results can still be trusted and the tributaries can be compared. Additionally, all data were collected only at one point in time during the day. This does not account for variances throughout the day caused by heavy rains or human activities. The data are simply a snapshot of something occurring at some point on the sampling day. Finally, land use was judged using knowledge of the area. Some of the land use classifications may not be correct.

This study acts as a foundation to extend the research further. The methods used had high

throughput and allowed a lot of meaningful data to be found quickly and effectively. Further research can utilize remote sensing to better categorize land use surrounding tributaries. Additionally, there are many creeks flowing into the Susquehanna River. This experiment only considered nine of them. Expanding the study to include more tributaries can allow more conclusions to be drawn with more support. Finally, by extending the experiment to include all seasons, differences between seasons can be observed.

In conclusion, there was a trend that showed higher DIN flux values in agricultural zones. These values show that these three tributaries contribute DIN to the Susquehanna River the most. If this trend is consistent for all tributaries of the Susquehanna River, tributaries in agricultural areas are undoubtedly contributing to high DIN flux values of the Susquehanna River for the Chesapeake Bay. If DIN continues to be high in agricultural areas, the increased flux values predicted by Horwarth *et al.* may come true (Horwarth *et al.* 2006). Eutrophication may also occur more and more (Kumar and Puri 2012).

As the population increases, effective food production becomes more and more important. Enough food for the population cannot be produced without utilizing nutrient-rich fertilizers (Kumar and Puri 2012). Animal farming also increases the amount of animal waste produced (Kumar and Puri 2012). Finally, as the population continues to grow, proper disposal of sewage and storm water becomes more and more important (Kumar and Puri 2012). It is important to fully understand major contributors to water pollution so they can be eradicated through BMPs and other legislature (Belval and Sprague 1999). These BMPs were effective during the 1980s-1990s, but the state of the world has changed. New research proves they are no longer effective (Horwarth *et al.* 2006), and changes must be made.

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<u>Appendix 1</u>

Aerial Views of Sampling Sites



Figure 8. Aerial view of Gardner Creek sampling site. Image taken from Google Earth.



Figure 9. Aerial view of Harvey's Creek sampling site. Image taken from Google Earth.



Figure 7. Aerial view of Hunlock Creek sampling site. Image taken from Google Earth.



Figure 8. Aerial view of Lackawanna River sampling site. Image taken from Google Earth.



Figure 9. Aerial view of Mill Creek sampling site. Image taken from Google Earth.



Figure 10. Aerial view of Shickshinny Creek sampling site. Image taken from Google Earth.



Figure 11. Aerial view of Sutton Creek sampling site. Image taken from Google Earth.



Figure 12. Aerial view of Tunkhannock Creek sampling site. Image taken from Google Earth.



Figure 13. Aerial view of Whitlock Creek sampling site. Image taken from Google Earth.

Sampling Sites



Figure 14. Gardner Creek sampling site.



Figure 15. Harvey's Creek sampling site.



Figure 16. Hunlock Creek sampling site.



Figure 17. Lackawanna River sampling site.



Figure 18. Mill Creek sampling site.



Figure 19. Shickshinny Creek sampling site.



Figure 20. Tunkhannock Creek sampling site.



Figure 21. Sutton Creek sampling site.



Figure 22. Whitlock Creek sampling site.