

BIOTIC AND ABIOTIC INDICATORS OF WATER QUALITY IN MUDDY RUN

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ABSTRACT

As many organisms need water for habitat and physiological functions, it is important to evaluate its quality. Water quality can be evaluated abiotically through alkalinity, dissolved oxygen, hardness, pH, and temperature measurements, or biotically through analyzing the amount of fecal coliform bacteria present in the water. Abiotic and biotic water conditions can create an unhealthy environment for both the aquatic and terrestrial biota, including humans. Types of macroinvertebrates found in water bodies serve as another biotic indicator of water quality. In fact, several biotic indices exist to classify stream water quality based on the macroinvertebrates residing in them. We hypothesized that water bodies with higher numbers of fecal coliforms would have fewer pollution intolerant macroinvertebrates. To test our hypothesis, we analyzed the water quality of Muddy Run, a stream in Huntingdon, Pennsylvania, by completing a qualitative study of the macroinvertebrates present in it. We also gathered abiotic water quality measurements to give us a broader understanding of the water quality including alkalinity, dissolved oxygen, hardness, pH, and temperature measurements. Our qualitative analysis of macroinvertebrates categorized Muddy Run as a moderately polluted stream, however we did not find a correlation between macroinvertebrate pollution tolerance and number of fecal coliform colonies. Overall, our abiotic parameters of Muddy Run reveal that its water quality is adequate for aquatic organisms, however, it is not suitable for human recreational use.

Keywords: Fecal coliforms, macroinvertebrates, water quality, pollution tolerance, Huntingdon

INTRODUCTION

Water quality is a very important topic, as many organisms need water for physiological functions and (or) habitat. Thus, it is important to evaluate water quality accurately. This is done using several common indicators of water quality, which include various biotic and abiotic factors.

Abiotic measurements of water quality include alkalinity, dissolved oxygen, hardness, pH, and temperature. Alkalinity affects the body of water's resistance to changes in pH; a high alkalinity helps prevent rapid changes in pH, protecting organisms in the water (Oram 2014a). Hardness is a measure of

calcium and magnesium ions dissolved in the water (Somridhivej and Boyd 2016). Moreover, calcium is necessary for aquatic organisms' bone and exoskeleton formation, blood clotting and other metabolic reactions (Wurts, W. A. 2014). Like alkalinity, hardness also affects pH since calcium is a base. pH is the concentration of hydrogen ions in the water, with lower pH's being more acidic, due to a higher concentration of hydrogen ions (EPA 2016). Dissolved oxygen describes the amount of oxygen that is in the water (Sánchez et al. 2007). The level of D.O. in the body of water can affect the survivability of aquatic organisms. Temperature affects water chemistry, especially DO; colder water holds more

DO than warm water (Cox 2003). Moreover, the flow can also influence the level of DO (the faster the flow is, the higher the DO level). Some taxa need more dissolved oxygen than others, but most need at least 4 to 7 mg/L (Cary Institute of Ecosystem Studies 2016).

One common biotic indicator for health of a body of water is the amount of fecal coliform bacteria in a water body. Although fecal coliforms generally do not cause disease themselves, they are good indicators of pollution, as most enter water bodies with fecal matter (EPA 2012). Fecal coliform contamination comes from runoff from lawns where pets are exercised, home septic systems that are not working properly, and fecal material from wild and domestic animals (Drohan, Sharpe and Smith 2004). Fecal coliforms are considered one of the primary indicators of water quality for recreational water in the United States (EPA 2012). Fecal contamination in recreational waters is associated with an increased risk of transmission of infectious diseases, such as gastrointestinal (GI) illness and less often identified respiratory illness, as harmful bacteria are often present if fecal coliforms are present (Office of Water 2012).

Macroinvertebrates are another indicator used to assess water quality, as these organisms are continuously exposed to the stream's conditions (Oleson 2013). Several biotic indices exist to classify the quality of streams based on the macroinvertebrates found in them (Oleson 2013). For instance, the presence of pollution-intolerant macroinvertebrates, such as mayflies, stoneflies and caddisflies, indicates healthy stream conditions. Consequently, we were wondering if there was a correlation between the number of fecal coliform colonies and the types of macroinvertebrates found in a stream. We hypothesized that areas of the stream with higher numbers of fecal coliform colonies would have fewer pollution intolerant macroinvertebrates than areas with a lower number of fecal coliform colonies.

We decided to study the quality of the water in Muddy Run, the stream that goes through Juniata College, by doing a qualitative study of the macroinvertebrates present in it, and by testing the water for the presence of fecal coliforms. To give us a better understanding of the water quality, we also gathered abiotic measurements of water quality, i.e. alkalinity, DO, hardness, pH, and temperature. We wish to test these hypotheses because the aquatic ecosystems can support many recreational activities, and we needed to make sure that the human population is not at risk of getting infectious diseases when they get in touch with this water.

We were expecting to find a worse quality of water upstream than downstream; the most upstream site is close to the East dormitories, and because of the littering we were expecting the water there to be more polluted, meaning we would likely find more fecal coliforms and less pollution-sensitive macroinvertebrates.

FIELD SITE

Muddy Run is located on the outskirts of the Juniata College campus in Huntingdon, Pennsylvania. It flows through residential homes northeast of campus and continues behind Weis Market. We divided the stream into four separate sites (Figure 1). We began our data collection at the East Apartments bridge heading towards Ellis Hall (Figure 2). We collected our data on the side of the bridge rather than underneath due to differences in sunlight and temperature. This section of Muddy Run consisted of mid-thigh high, murky water with darkened leaf matter and litter along the edges. Besides macroinvertebrates, we observed Green Frogs, *Lithobates clamitans*, and blacknose dace, *Rhinichthys atratulus*. Sediments in the stream comprised mainly of gravel and silt.

The second site was located further downstream behind the baseball field. This section of the stream was extremely shallow with small boulders, large cobbles, gravel, and sand (Figure 3). The flow of water created ripple marks in the sediment underwater. Vegetation consisted mainly of watercress along the edges of the stream and moss on the larger rocks.

The third examined section of Muddy Run was located at the end of the baseball field (Figure 4a). We noticed a corrugated drainage pipe came from the side of the stream (Figure 4b). This section also consisted of boulders and cobbles along the sides and floor of the stream. Vegetation mainly included algae with no other observed aquatic flora. Other noticed biota included the blacknose dace, *Rhinichthys atratulus*, and tadpoles of the Green Frog, *Lithobates clamitans*.

The last site we examined was located downstream of the bridge behind Weis Markets (Figure 5a). A small building with bathrooms was located next to the stream with a drainage tunnel draining into the stream (Figure 5b). Similar to our second site, our fourth site consisted of gravel and sand. Vegetation included small patches of watercress and algae. The water is visibly clear enough to see the ripple marks created in the sand. We also found a blacknose dace, *Rhinichthys atratulus*.

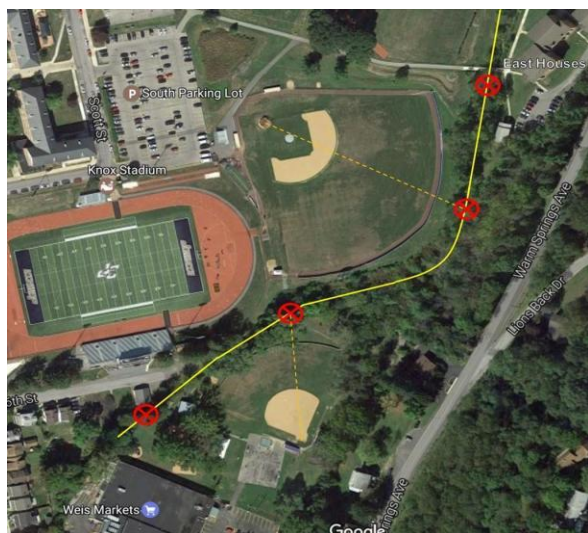


Figure 1. Map of Muddy Run indicating the locations of our sites, beginning at East Houses and ending downstream at the bridge behind Weis Markets. The yellow line indicates Muddy Run, and the red crossed circles represent our sampling sites.

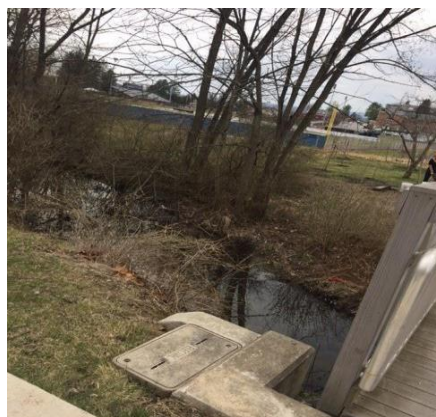


Figure 2. Site 1 at East Houses.

Figure 3. Site 2 behind the baseball field.

Figure 4a. Site 3 at the end of the baseball field.



Figure 4b. Site 3 Corrugated Drainage Pipe

Figure 5a. Site 4 behind Weis Markets.



Figure 5b. Site 4 Drainage Tunnel.

METHODS AND MATERIALS

On March 25, we collected macroinvertebrates and measured fecal coliforms and hardness for sites 1 and 2. The weather conditions on this date were cloudy and a high of 71°F, according to

the weather channel iPhone app. On March 29, we collected macroinvertebrates and measured the pH, hardness, and fecal coliforms for sites 3 and 4. On the same date, we measured the alkalinity for all four sites. The weather was sunny with a high of 59°F. On April 2, we measured temperature and dissolved oxygen for all sites. The weather conditions on April 2 were 60°F and partly cloudy with some spurts of sun. On April 12, we collected macroinvertebrates for all sites as well as the pH measurements for sites 1 and 2. The weather conditions on April 12 were sunny and 67°F. We determined the number of fecal coliform colonies in the petri dishes on March 26 for sites 1 and 2 and on March 30 for sites 3 and 4.

Alkalinity, dissolved oxygen, hardness, temperature, pH, and fecal coliforms were measured for each of the four sites. We took two water samples at each site by rinsing two cylindrical containers in the stream water three times prior to collecting and sealing the water. Using HACH water test kits, we determined alkalinity and hardness within twenty-four hours of the samples being taken (Hach, Loveland, Colorado). We determined pH using a Markson Digital pH meter model 88 (Markson, Henderson, NC). Dissolved oxygen and temperature measurements were taken with a YSI dissolved oxygen and temperature probe model 55 (YSI, Yellow Springs, Ohio). Temperature and dissolved oxygen were measured three times at each site then averaged. Likewise, our results for alkalinity, hardness, and pH are averages of the two water samples for each site.

We quantified the number of fecal coliform colonies using Coliscan Easygel kits, following the recommended protocol (Microbiology Laboratories, Goshen, IN). We used 1 mL of water from each sample and converted the resulting number of fecal coliform colonies to number of colonies per 100 mL after allowing the samples to incubate for twenty-four hours at 35°C. Then we averaged the number of fecal coliforms for each site.

At each site, we took two qualitative samples of macroinvertebrates; we spent thirty minutes collecting them, using sieves and nets. We later identified them using a dichotomous key (Kellogg 1994), which gave common names and orders for most of the macroinvertebrates we found. Then we classified each of the identified macroinvertebrates by pollution tolerance, according to a biotic index (Sharpe, Kimmel, and Buda).

We used the Kruskal Wallis test to determine if the numbers of fecal coliform colonies per site were significantly different and if the number of taxa found for each pollution tolerance varied among the

sampling sites for the two qualitative assessments. All Kruskal Wallis tests were done with 3 degrees of freedom. Additionally, we plotted the number of fecal coliforms with the number of taxa for each pollution tolerance for the different sites to see if there was a correlation between them, and we used a two-tailed linear regression t-test to see if the correlations were significant, using a TI-83 graphing calculator.

RESULTS

Many taxa were present in at least two sites (Table 2 and Table 4). Consequently, most of the streams were found to be moderately polluted, except for site 3, which was considered grossly polluted according to the results of our first qualitative assessment and Sharpe, Kimmel, and Buda's biotic index (Table 3 and Table 5). Notably, crayfish, a pollution sensitive taxon, were only found at sites 1 and 4, and mayflies, another pollution sensitive taxon, were only found at site 2.

However, the number of taxon found for each pollution tolerance was not statistically different among the sampling sites for either qualitative assessments. The H test statistic for the Kruskal Wallis test on the first qualitative assessment was 0.57, with a p value of 0.903. Similarly, the H test statistic for the Kruskal Wallis test on the second qualitative assessment was 0.667, with a p value of 0.881.

Most of the scatterplots with number of fecal coliforms and number of taxa had a negative slope for the regression line (Figures 8, 9, 11, 12, and 13). However, some of those regression lines had very low R^2 values, meaning the line captured little of the variation among the sampling sites (Figure 9 and Figure 13). Moreover, none of the R^2 values were greater than 0.3253 (Figure 10 and Figure 12). The differences in the number of fecal coliform colonies per 100 mL of water were not found to be significant among the sampling sites, as the H test statistic was 4.019, and the p value was 0.259 for the Kruskal Wallis test. Moreover, none of the correlations were significant. Moreover, the number of fecal coliforms did not increase or decrease going downstream, instead they varied by section starting at the East Houses with 400 colonies per 100 mL and sites two, three, and four with 100, 300, and 350 colonies per 100 mL respectively (Table 1).

According to our abiotic water quality measurements the temperature throughout the studied section of Muddy Run remained constant (Table 1). However, the concentration of dissolved oxygen

DOI

increased moving downstream from the first site at East Houses to the fourth site at Weis Markets. The concentration of dissolved oxygen in Muddy Run is well above Environmental Protection Agency's, EPA, standard for Pennsylvania streams (Figure 14). The pH of the stream varied from site to site, but remained within EPA standards (Figure 15). The alkalinity of the sites also varied between site locations. However,

the alkalinity of all four sites are under the EPA's minimum standard for alkalinity in Pennsylvania streams (Figure 16). The hardness for the sites also varied, however, the fourth site at Weis Markets had a noticeably lower measurement of 190 mg/L of calcium and magnesium, whereas the first three sites had a hardness of 230, 210, and 220, reported in site order (Figure 17).

Table 1. Water quality measurements for the four Muddy Run sampling sites.

Sampling Site	Alkalinity (mg/L of CaCO ₃)	Dissolved Oxygen (ppm)	Fecal coliforms (Colonies per 100 mL)	Hardness (mg/L of Ca and Mg)	Temperature (°C)	pH
Site 1	11.2	11.87	400	230	13.1	6.92
Site 2	9.6	12.50	100	210	12.9	7.42
Site 3	11.0	12.72	300	220	12.9	7.31
Site 4	10.4	12.76	350	190	12.9	7.41

Table 2. Macroinvertebrate taxa identified in the first qualitative sample for the four Muddy Run sampling sites in Huntingdon, Pennsylvania. Common names are in parentheses.

Taxon	Site 1	Site 2	Site 3	Site 4
Order: Amphipoda (amphipod)		X	X	X
Class: Bivalvia Family: Corbiculidae (asian clam)			X	
Order: Decapoda (crayfish)	X			
Order: Diptera Family: Chironomidae (non-biting midge)	X	X		X
Class: Gastropoda Subclass: Prosobranchia (operculate snails)	X	X	X	X
Class: Gastropoda Subclass: Pulmonata (non-operculate snails)	X	X	X	X
Order: Hemiptera Family: Gerridae (water strider)				X
Class: Hirudinea (leech)	X	X	X	
Order: Isopoda (isopod)		X		X
Order: Odonata (damselfly)	X	X	X	X
Order: Odonata (dragonfly nymph)	X			X
Class: Oligochaeta (aquatic worm)		X		X
Class: Turbellaria (flatworm)		X	X	X

Table 3. Pollution sensitivities for the first qualitative sample for the four Muddy Run sampling sites in Huntingdon, Pennsylvania (Sharp, Kimmel, and Buda).

DOI

Sampling Site	Pollution sensitive macroinvertebrates (number of taxa)	Moderately tolerant macroinvertebrates (number of taxa)	Pollution tolerant macroinvertebrates (number of taxa)	Stream Classification
Site 1	1	2	3	Moderate pollution
Site 2	0	3	4	Moderate pollution
Site 3	0	2	3	Gross pollution
Site 4	0	4	4	Moderate pollution

Table 4. Macroinvertebrate taxa identified in the second qualitative sample for the four Muddy Run sampling sites in Huntingdon, Pennsylvania. Common names are in parentheses.

Taxon	Site 1	Site 2	Site 3	Site 4
Order: Amphipoda (amphipod)	X	X	X	X
Class: Bivalvia Family: Corbiculidae (asian clam)				
Order: Coleoptera Family: Haliplidae (crawling water beetle)	X	X	X	X
Order: Hemiptera Family: Corixidae (water boatman)	X	X		
Order: Decapoda (crayfish)	X			X
Order: Diptera Family: Chironomidae (non-biting midge)	X	X	X	X
Order: Ephemeroptera (mayfly)		X		
Class: Gastropoda Subclass: Prosobranchia (operculate snails)	X	X		
Class: Gastropoda Subclass: Pulmonata (non-operculate snails)	X	X	X	
Order: Hemiptera Family: Gerridae (water strider)	X	X		X
Class: Hirudinea (leech)	X	X		
Order: Isopoda (isopod)		X	X	X
Order: Odonata (damselfly)	X	X	X	X
Order: Odonata (dragonfly nymph)	X	X	X	X
Class: Oligochaeta (aquatic worm)	X	X	X	X
Class: Turbellaria (flatworm)		X	X	X

Table 5. Pollution sensitivities for the second qualitative sample for the four Muddy Run sampling sites in Huntingdon, Pennsylvania (Sharp, Kimmel, and Buda).

DOI

Sampling Site	Pollution sensitive macroinvertebrates (number of taxa)	Moderately tolerant macroinvertebrates (number of taxa)	Pollution tolerant macroinvertebrates (number of taxa)	Stream Classification
Site 1	1	3	7	Moderate pollution
Site 2	1	4	8	Moderate pollution
Site 3	0	4	4	Moderate pollution
Site 4	1	4	4	Moderate pollution

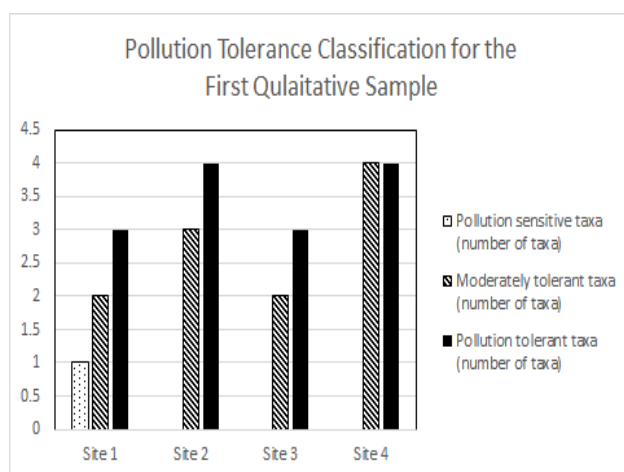


Figure 6. Number of macroinvertebrate taxa for each pollution tolerance classification for the first qualitative sample (Sharp, Kimmel, and Buda).

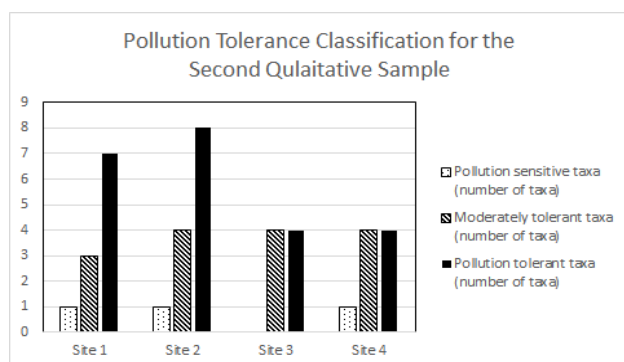


Figure 7. Number of macroinvertebrate taxa for each pollution tolerance classification for the second qualitative sample (Sharp, Kimmel, and Buda).

Fecal Coliforms and Tolerant Taxa (First Qualitative Assessment)

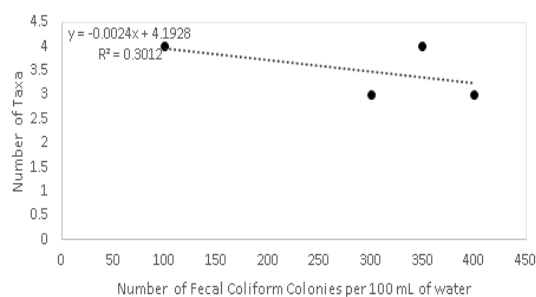


Figure 8. Correlation analysis between number of pollution tolerant macroinvertebrates (identified to class or order) and number of fecal coliform colonies for the first qualitative assessment. $p = 0.45$.

Fecal Coliforms and Moderately Tolerant Taxa (First Qualitative Assessment)

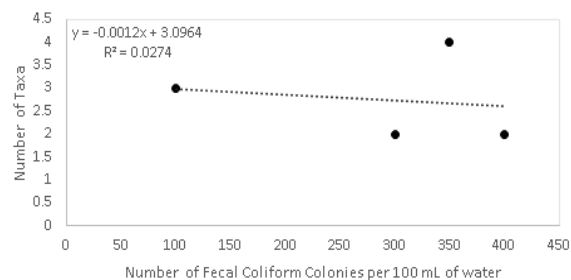


Figure 9. Correlation analysis between number of moderately tolerant macroinvertebrates (identified to class or order) and number of fecal coliform colonies for the first qualitative assessment. $p = 0.83$.

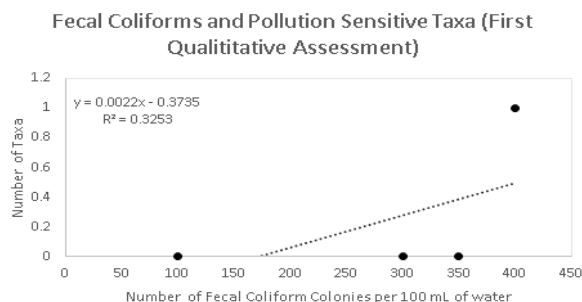


Figure 10. Correlation analysis between number of pollution sensitive macroinvertebrates (identified to class or order) and number of fecal coliform colonies for the first qualitative assessment. $p = 0.43$.

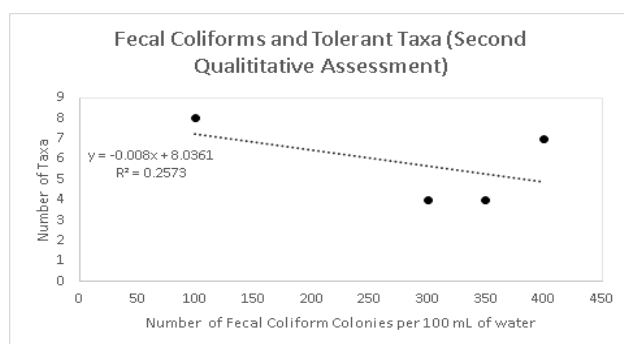


Figure 11. Correlation analysis between number of pollution tolerant macroinvertebrates (identified to class or order) and number of fecal coliform colonies for the second qualitative assessment. $p = 0.49$.

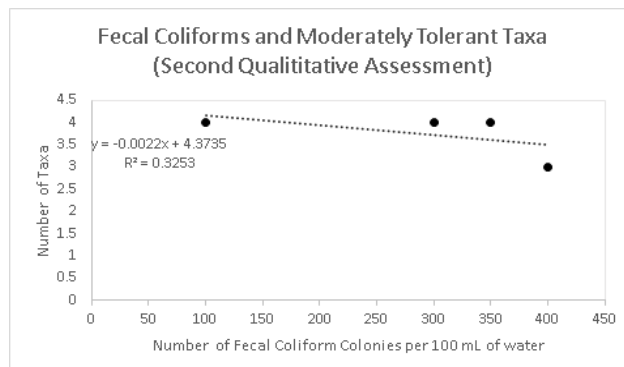


Figure 12. Correlation analysis between number of moderately tolerant macroinvertebrates (identified to class or order) and number of fecal coliform colonies for the second qualitative assessment. $p = 0.43$.

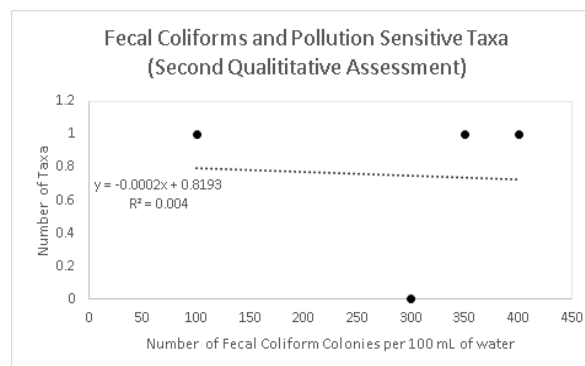


Figure 13. Correlation analysis between number of pollution sensitive macroinvertebrates (identified to class or order) and number of fecal coliform colonies for the second qualitative assessment. $p = 0.93$.

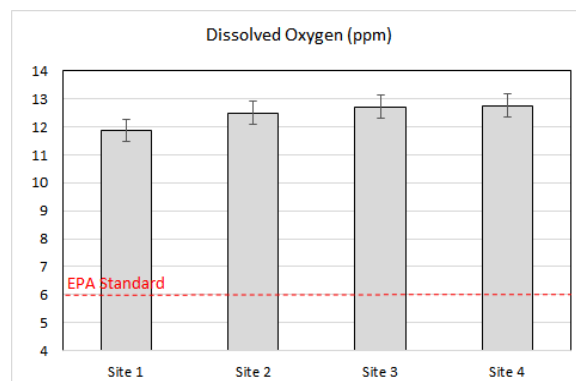


Figure 14. The dissolved oxygen measurements in parts per million for each of the four sites of Muddy Run compared to the EPA's Water quality standard for dissolved oxygen according to Chapter 93 of the Pennsylvania Clean Streams Law (EPA 1971).

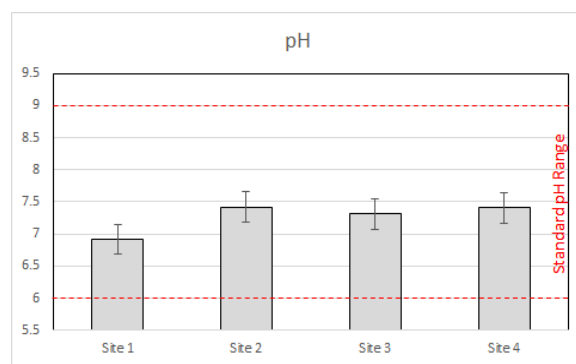


Figure 15. The pH measurements for each of the four sites of Muddy Run. The red dotted lines represent the EPA's Water quality standard range of pH according to Chapter 93 of the Pennsylvania Clean Streams Law (EPA 1971).

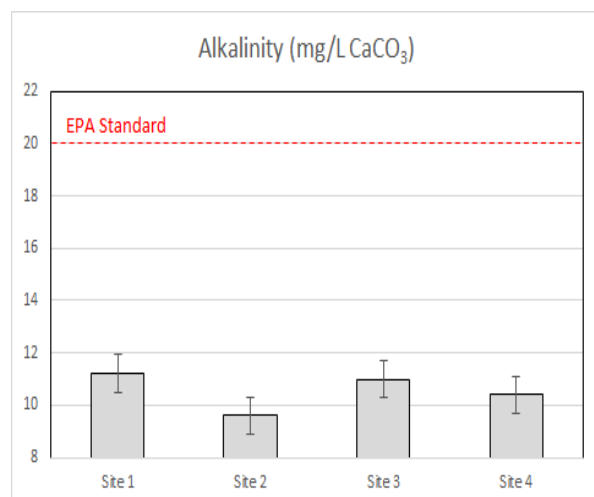


Figure 16. The alkalinity measurements in mg/L of calcium carbonate for each of the four sites of Muddy Run compared to the EPA's Water quality standard for alkalinity according to Chapter 93 of the Pennsylvania Clean Streams Law (EPA 1971).

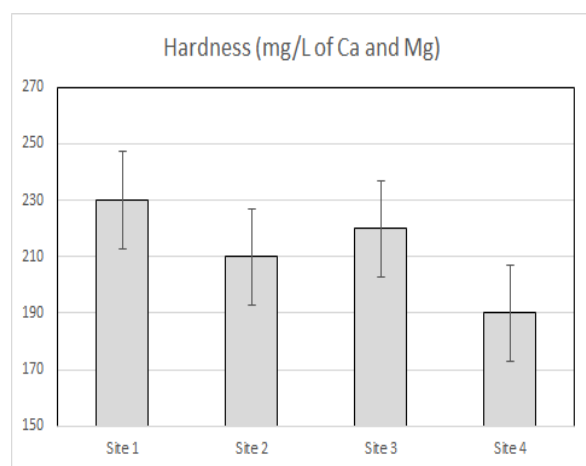


Figure 17. The hardness measurements in mg/L of calcium and magnesium for each of the four sites of Muddy Run.

DISCUSSION

As we analyzed our data, we realized that some factors may have affected our results. One major factor could be the dates and times that we collected our data. For our initial macroinvertebrate collections on March 25 and 29 we also collected water samples for the fecal coliforms on the same days. However, weeks later on April 12, we did not determine the number of fecal coliforms that correlated to the second sample of macroinvertebrates collected that day. With the first and second macroinvertebrate collections being half a month apart, it is possible that the water composition of Muddy Run changed as we moved

further into spring. Perhaps, if we had collected abiotic water quality measurements for both macroinvertebrate collections we would have had more data to analyze.

Another factor that could have given us a better sense of the water quality of Muddy Run is our macroinvertebrate collection method. We collected macroinvertebrates qualitatively, in which we wrote down the types of taxa we found at each site of the stream. Qualitative macroinvertebrate collection provides us only with the types of taxa in Muddy Run, and does not describe which taxa are most abundant. If there were a higher abundance of pollution sensitive taxa in comparison to the moderately and pollution tolerant taxa at site 1, then we would have a better picture of the water quality. Using a quantitative macroinvertebrate collection method would have provided us with the abundances of taxa within the different pollution sensitivities, which could potentially give us different conclusions.

As shown in the Results section, we did not find any significant correlation between the number of fecal coliform colonies and the types of macroinvertebrates found in a stream. Based on our findings, it does not seem like the concentration of fecal coliforms in the water of Muddy Run had a significant influence on the types of macroinvertebrates living there. However, these results might be due to the fact that we did not take enough samples in the stream, as well as use enough sample for the Coliscan Easygel kit, and our sampling sites did not have a large range of water qualities. Another study that used both fecal coliforms and macroinvertebrates; Mcnett, Hunt, and Osbourne found a negative correlation between fecal coliforms and water quality based on the macroinvertebrates for one of their three stream classifications (2010). That group had the greatest range in stream water qualities, giving evidence that a lack of sufficient variation among our sample sites may have affected our results.

Our results for fecal coliform colonies were interesting, as they showed Muddy Run should not be used for drinking or recreation. For recreational waters, the mean number of fecal coliform colonies should not exceed 200 colonies per 100 mL of water, for five samples over a thirty-day period (EPA 1986a). Except for site 2, which had 100 colonies per 100 mL of water, all of the other sites had more than 300 colonies per 100 mL of water; therefore, we can tell that Muddy Run does not have a good concentration of fecal coliforms for humans, especially for drinking. According to Swistock, Clemens, and Sharpe, it is unsafe to drink water with any detectable amount of fecal coliforms (2017).

The qualitative assessment indicated that Muddy Run has decent water quality since several

species appear to be surviving and thriving. Interestingly, this finding contrasts with the results of a previous study by Juniata students that concluded Muddy Run was polluted based on the absence of some pollution intolerant taxa and the abundance of chironomid fly larvae, a pollution tolerant taxa (Chilcote et al. 1998). Therefore, our qualitative assessments give some evidence that Muddy Run's water quality is better now than it was previously. The mayfly we found is especially notable since it is a pollution sensitive taxon (Sharpe, Kimmel, and Buda) that was not previously found in Muddy Run (Chilcote et al. 1998).

Our abiotic measurements also indicated that Muddy Run is a fairly healthy stream. All our pH values are around 7 so we can consider that the water of our stream is neutral, which is a very good pH to sustain life in a stream, as the pH of streams is normally between 6.5 and 8.5 (Oram 2014b). Therefore, a pH outside of that range could be evidence of pollution. The alkalinity we found for Muddy Run is between 9.6 and 11.2 mg/L of CaCO₃; it is smaller than the standard minimum alkalinity of the streams in PA, which is 20 mg/L. Consequently, even though the water of Muddy Run has a neutral pH, the water is not very resistant to changes in pH, so the pH could change rapidly. The effect of changes of pH could be dramatic for aquatic organisms, especially for those that have calcium exoskeletons.

Temperature also affects aquatic ecosystems. With temperatures remaining constant throughout the examined sections of Muddy Run, we inferred that temperature did not play a large part in the differing characteristics of the stream sections, including the stream classifications in Table 3 and 5. Similarly, the dissolved oxygen values we found were all around 12 mg/L; however, it is noticeable that the concentrations increased heading downstream. During our data collection, we observed that the flow of Muddy Run also increased as we moved further downstream. With faster waters being able to diffuse more atmospheric oxygen into water than slower waters, it explains why the dissolved oxygen concentrations increased going downstream when temperature is relatively constant. The minimum level of dissolved oxygen needed is between 4 and 7 mg/L for most aquatic organisms (Cary Institute of Ecosystem Studies 2016). Therefore, Muddy Run has a suitable level of DO to sustain life.

The hardness we measured in Muddy Run is around 190 and 230 mg/L, which is within the acceptable range for hardness in fish cultures, 63-250 mg/L (Wurts 2014). The EPA does not have an acceptable range for hardness, but it notes that a higher

hardness can reduce the effects of toxic heavy metals in an aquatic ecosystem (EPA 1986b).

Overall, the abiotic parameters of Muddy Run are pretty good and adequate for the survival and thriving of many aquatic organisms. The main issue is that the alkalinity is pretty low and that the pH could change rapidly, for instance, the pH could dramatically decrease if acidic rain were to occur. Our qualitative index provides good evidence that Muddy Run's water quality has improved, showing the value of re-examining an area after several years, instead of just assuming quality remained unchanged. Additionally, the conflicting indications given by the qualitative assessment and the fecal coliform results illustrate that a variety of measurements and factors must be considered in order to evaluate water quality accurately, contributing to the wealth of studies that have previously been done on water quality.

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