Assessing variability in water quality parameters along Two Mile Run in Venango County, northwestern Pennsylvania, to monitor unconventional shale gas extraction impacts on stream quality

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Hydraulic presents potential risks to surrounding water supplies if operations and water bodies are not properly or regularly monitored. Headwater streams are of most importance when considering where to monitor mainly because they contain the basis of the food web for biota downstream (including humans). This study assessed the variation in water quality parameters along a stream in northwestern Pennsylvania. The study took place in a watershed that contains an horizontal hydraulic fracturing shale gas well. The goal of this study was to detect change in water chemistry and macroinvertebrate assemblages between sites above (upstream) the well and sites below (downstream from) the well and determine if change was caused by an impact from the well, some other land disturbance, or part of the natural flow of materials and biota of the stream. Abiotic indicators showed a significant difference in TDS, conductivity, and pH between upstream and downstream sites. These results, along with an assessment of the surrounding land cover and riparian zones, indicated some impact by the well or other land disturbance. Macroinvertebrate assemblage samples showed a presence of extremely sensitive taxa in both upstream and downstream sites. Although the stream is currently considered healthy, continued biomonitoring is recommended to ensure continued stream health.

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Introduction

Importance of Streams

Seventy percent of the Earth's surface is water. The majority of that is salt water and only about 2.5% of it is freshwater. Of that freshwater, over half of it is inaccessibly locked in glaciers, while the rest of it is mostly inaccessible to humans in soil moisture and deep groundwater aquifers. Only about 1% of freshwater is accessible for human use (Postel et al., 1996). This accessible water is found in shallow groundwater, lakes, ponds, rivers and streams. According to the United States Environmental Protection Agency (2013a), there are more than 3.5 million miles (5.6 kilometers) of rivers and streams in the U.S. Around 53% of those miles of streams, in the continental U.S., are headwaters (United States Environmental Protection Agency, 2013b).

Streams have a variety of functions that benefit many different organisms. They provide habitats for a diverse range of biota, from microorganisms, macroinvertebrates and fish to humans using water as a resource and recreation. The headwaters of a stream are rarely used by humans for recreation, but play an important part in our lives. Typically, recreation such as boating, fishing, or swimming occurs in higher order streams where water is deep enough for these activities to be successful or in lakes created by damming. Streams are categorized based on how much water and materials are passing through them. Therefore, a higher order streams (fourth-order or higher) are larger than a first-, second- or third-order stream (which are all classified as headwaters). If headwater streams are in any way contaminated or changed, the effects accumulate as the contaminant travels downstream (Allan and Castillo, 2007). The River Continuum Concept is a model used to classify streams and rivers and is based on the equilibrium of physical, chemical and biological parameters of a flowing body of water. It is therefore used to predict where certain biological communities can be found along the stream from the headwaters to mouth. According to this concept, if biota is affected in the headwaters, the biota in the rest of the stream will also be affected because the energy supplied to the food web of the stream comes from the headwater in the form of primary production. If the biota responsible for providing that energy input, such as detritivores, are affected in a way that they are not able to provide that energy, lower downstream stretches will also be affected (Allan and Castillo, 2007). Any large addition of materials (nutrients, chemicals, sedimentation, excessive additions of water, etc.) to headwater streams cause changes in higher order streams and rivers.

Disruptions like these can alter the biology and integrity along the length a stream from headwater to mouth. These changes impact biota at all levels in the food web, including humans. Headwater streams must be protected in order to ensure human access to safe drinking water, recreation and the conservation of biological diversity.

Threat of Hydrofracking

Hydraulic fracturing, commonly called "hydrofracking" or just "fracking," is an increasingly popular method for extracting natural gas. This process has the potential to contaminate or disrupt streams, especially if the well is located near surface water. Natural gas companies use vertical and horizontal wells to capture natural gas, but horizontal drilling is presently the most popular natural gas extraction method. To create a horizontal (unconventional) natural gas well, a vertical well is drilled through bedrock until it reaches the shale layer, the layer of rock that contains the natural gas, and then the drill moves horizontally across the shale bed. Once the hole is made, a well bore is placed inside with a casing to prevent leakage of initial fluids and flowback. A large amount of fluids consisting of water, proppants, and other additives are pumped at a high pressure through the well bore. This high-pressured fluid causes fractures in the shale, which the proppant flows into. The proppant consists of sands and other particles that keep the fractures open so gas can be released and captured (Weltman, 2013).

The potential threat to streams comes when pressure from the bedrock causes flowback water back up to the surface through the well bore. This process occurs naturally and the gas is extracted after the flowback water is out of the well. Flowback water contains some of the chemicals and materials in the fluids that were pumped into the well, but also contain natural minerals and materials from the rock itself. The other materials consist of brine (salts), metals, and hydrocarbons (U.S. Environmental Protection Agency, 2014 and Maloney and Yoxtheier, 2012). Flowback water is usually contained on site in retention ponds/tanks. On-site retaining ponds threaten to leak or runoff if there is heavy rainfall. These retaining ponds usually are not covered in PA and are not required to have multiple linings. Cracked casing around the well threatens to leak into water sources. In a study assessing the well casings and cement impairments in Pennsylvania, Ingraffea et al. (2014) found that horizontal unconventional shale gas wells were more likely to display structural integrity problems than vertical conventional

wells. The most common issues were defective cement and casing, improper installment of casings and sustained build-up of natural gas in the casing. Most cases of water contamination from hydraulic fracturing have been traced back to faulty cement and well casings, not the actual extraction method.

There are federal and state regulations in place to protect stream health and prevent negative impacts on human health regarding clean water (Clark et al., 2012). These regulations were created for the type and amount of chemicals used in fracking fluids, the method of waste management and disposal, and proper wastewater treatment (Clovis and Pifer, 2009). There are many small gas companies in operation, which can make it difficult for state or federal agencies to manage routine protocol checks. If a problem is detected with their methods, the regulation committee, whether it is state (PA Department of Environmental Protection) or federal (U.S. EPA), will take action. Another issue with regulation and stream monitoring is that many of these agencies do not have the resources to regularly monitor streams and rivers around gas extraction operations. Under the Pennsylvania Oil and Gas Act, drilling companies are not required to monitor the water quality of nearby streams or rivers before or after the drill a well (PA Code, 1987), they are encouraged to monitor for their own benefit.

Natural shale gas extraction methods not only pose potential risk of water chemistry changes, but also pose stream sedimentation risks. Temporary roads built for the transportation of materials to and from the well pad site are usually made of compact dirt. These roads are prone to erosion when it rains. Sediment gets washed down roads or travels through ditches on the sides of roads and enters the stream directly as surface runoff. Sedimentation can destroy stream habitat and affect numerous species of biota such as fish, mussels and aquatic insects (macroinvertebrates). Specifically, sedimentation can change the abundance and diversity of benthic macroinvertebrates (BMIs). Sediment fills interstitial spaces between rocks where BMIs are usually found, causing increased drift to new habitats downstream and changes in feeding behavior. Sedimentation also leads to a decrease in density and diversity of BMIs, and causes them to move from riffle habitat to other habitats in the stream, or causes them to abandon the stream altogether (Roy et al., 2003).

These risks have the potential to threaten the integrity of nearby streams. Therefore, it is important to regularly monitor streams in close proximity to natural gas wells.

Importance of Baseline Data and Biomonitoring

Baseline data are very important to have in any field of research, especially environmental protection and conservation. Baseline data document environmental conditions before significant change occurs, so that change, usually due to anthropogenic sources, can be identified, measured and assessed. In some studies, however, true baseline data are impossible to collect because there is already an anthropogenic change to the natural environment. In my study, such changes might include active or abandoned gas or oil wells, roads, farms and/or residential homes or other urban development. Bodies of water in close proximity to agricultural land may have increased amounts of nitrogen and phosphates due to fertilizer runoff. Unnaturally large amounts of these synthetic fertilizers can cause eutrophication in nearby bodies of water. Eutrophication occurs when an addition of nutrients from the fertilizers causes algal blooms. When the algae dies and sinks to the bottom of the stream, microbes breakdown organic matter, using up the oxygen in the water during the process of decomposition. Most aquatic life cannot live in the hypoxic environment and they either die off or move to another reach in the stream. All of the types of land use and land cover types mentioned above can disrupt the natural state of a stream. It is still important to regularly monitor streams in close proximity of potential threats to their biological integrity despite a lack of true control. In doing so, it is important to consider all types of land use and land cover surrounding a stream to determine why fluctuations in water quality parameters exist. Although this is not baseline data, it is vital information that will be useful in determining any further changes to the stream.

Measuring changes is where the practice of biomonitoring comes into play. Biomonitoring practices are used to detect the health of a stream. Some techniques used in biomonitoring include water chemistry tests such as dissolved oxygen, turbidity, conductivity, pH, total dissolved solids, and levels of nitrogen and phosphorus. Assessing macroinvertebrates assemblages (abundance and diversity of taxa) is another important technique used in biomonitoring. Some Orders of macroinvertebrates are extremely sensitive to changes in water chemistry and habitat disturbance. The most sensitive Orders include Ephemeroptera (mayflies),

Plecoptera (stoneflies) and Trichoptera (caddisflies). These are referred to as "EPTs." Other sensitive groups include Order Megaloptera (dobsonflies and alderflies) and Phylum Mollusca (clams, mussels and snails). Groups that are more tolerant of environmental changes include Order Diptera (true flies) and Family Tubificidae (tubifex worms) of Order Annelida. The condition of a stream can therefore be determined by sampling macroinvertebrates. Considering the riparian zone around a stream also helps to determine and mitigate possible non point-source pollution. Riparian zones of good quality have a few meters thick of vegetation, usually grasses, shrubs and trees. The roots of the vegetation not only hold the stream banks together to help prevent erosion, but they also filter runoff from the land beyond the stream. Poor riparian zones allow sedimentation and runoff possibly containing excess nutrients or other chemicals to run directly into the stream. Riparian zones tie directly to land cover and land use surrounding a stream. The land cover determines the amount of runoff flowing into a stream. For example, a field of row crops would have less water uptake by roots than forested land. Land use also determines the amount of runoff (amount of impervious surfaces) as well as the types of pollution (fertilizers, salts, contaminated water from natural resource extraction, sewage or other organic matter). All of these factors are important to consider when collecting baseline data and determining the health of a stream or watershed.

Purpose of Study

This study assesses the biological integrity of Two Mile Run, located in Venango County in northwestern Pennsylvania (Figure 1). Biological integrity is commonly defined as the ability of an ecosystem to support a balanced community of organisms with species comparable to those of natural habitats with the region in regards to composition, diversity and functionality (Karr and Dudley, 1981). Two Mile Run is a headwater stream in the Two Mile Watershed, which is a sub-watershed of the Middle Allegheny Tionesta Watershed (Figure 1). This stream is of importance because it flows through the Two Mile County Park into Justus Lake, a reservoir, and flows into the Allegheny River further down in the sub-watershed. The majority of the sample sites used in this study are located in Two Mile County Park. The park is primarily for recreation and offers hunting, fishing, hiking, picnicking, and mountain biking opportunities. Justus Lake contains warm and cold-water species of fish such as trout, bass, muskellunge, walleye and pan fish. Based on the idea of synergism, any added substance to the stream could subsequently cause

greater change to Justus Lake and affect the recreational activities that it offers (Dijk et al., 2013). The same idea applies to the effects on the Allegheny River further downstream. The Allegheny River is the source of drinking water for many towns and cities along the river. Major contamination to headwater streams could affect many people downstream. Two Mile Run was chosen for this study because an active, unconventional horizontal natural gas well is located in the watershed. It is also in Trout Unlimited Conservation Success Index monitoring zone (described further in the methods section of this paper), which takes into consideration present and future shale gas development areas as well as wild and native trout populations.

The objectives of this study were (1) to assess water quality parameters to detect variability along Two Mile Run, (2) observe abiotic and biotic variation between sites that lie above the well in the watershed and sites that lie below the well, and (3) to determine whether variability is due to the natural stream system, shale gas well, or other land cover/land use cause. The hypotheses for this study are (1) water chemistry parameters (TDS, turbidity, conductivity and pH) will increase in downstream sites and (2) macroinvertebrate diversity will show worsened stream quality downstream.

Past Research on Two Mile Run

Jillian D'Amico performed a similar study on Two Mile Run in 2013. The purpose of her study was to collect and analyze baseline data of stream health upstream and downstream of deep shale gas wells. D'Amico was focusing on well construction impacts to the streams. Changes in water chemistry and macroinvertebrate assemblages were observed as the construction progressed in December 2012, and February and March 2013.

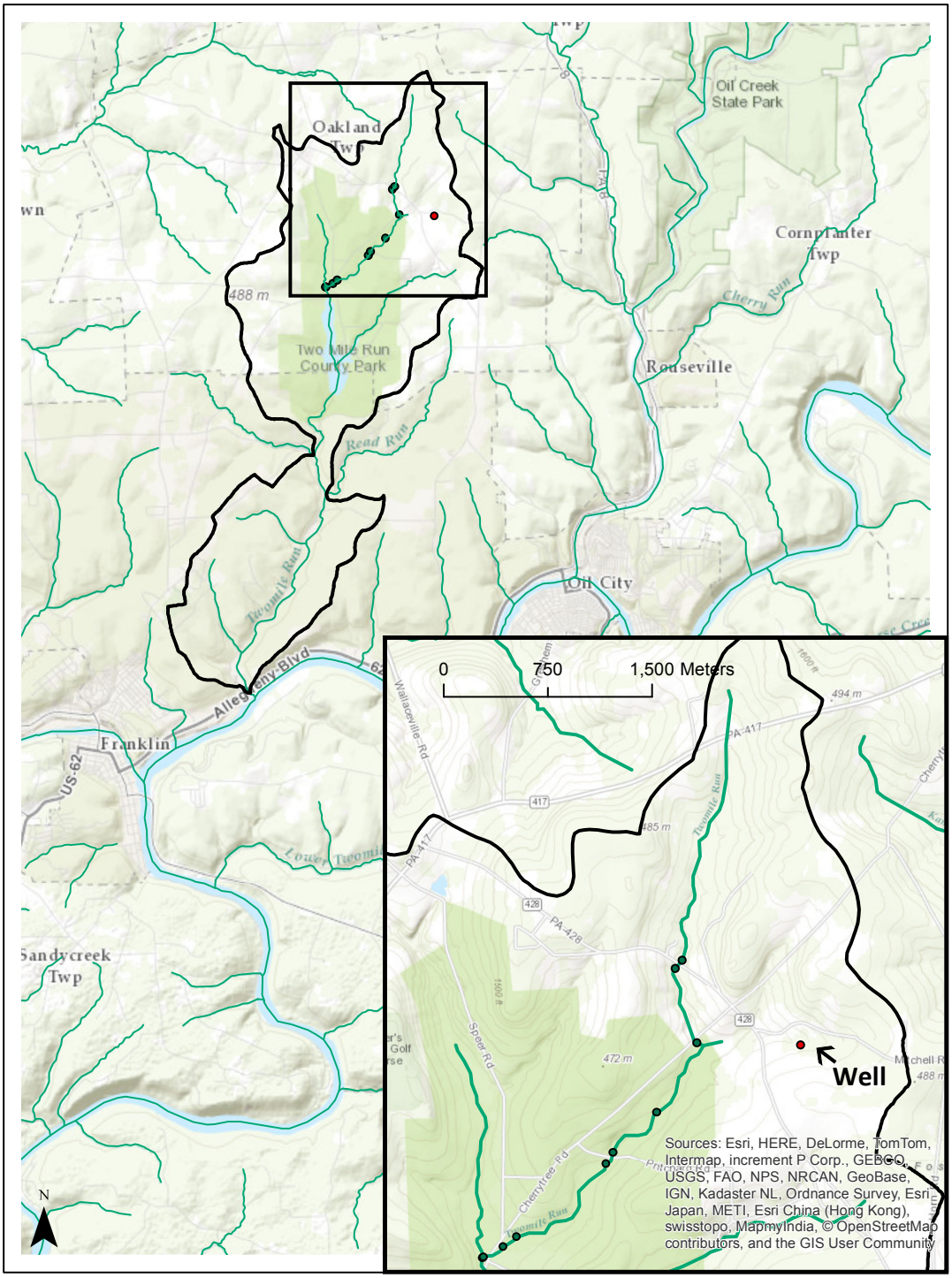


Figure 1. Topographic map of Two Mile watershed showing an inset map of the study area with locations of sample sites. Notice most downstream sites are in Two Mile County Park.

Methods

Site Selection

Table 1. GPS coordinates of the sample sites and well location.

Site	GPS Coordinates
1	41.505311, -79.755733
2	41.504934, -79.756135
3	41.500002, -79.754474
4	41.494529, -79.758676
5	41.493055, -79.761800
6	41.492569, -79.761991
7	41.487171, -79.770789
8	41.486839, -79.771352
9	41.486178, -79.773180
10	41.486170, -79.773239
Carter 4H unit	41.498161, -79.746579

For this study, ten sample sites were chosen along Two Mile Run in the town of Franklin, Venango County, northwestern Pennsylvania. Each site's GPS coordinates were recorded (Table 1). Sites 3-10 are located in Two Mile County Park, Franklin, PA.

Data were collected along a 3,000 meters stretch of Two Mile Run from the location where it intersects Route 428 to a location before the stream enters Justus Lake (Figure 1). The sites were chosen based on the types of land use (i.e. agriculture, development, recreation, etc.) and land cover (i.e. forest, field, open water, etc.) along a stretch of stream (Figure 2). For example, sites were chosen upstream and downstream from roads, farmed land and a pavilion area containing a restroom with pit toilets. Variability was assessed in the water quality parameters along the stream and then compared to surrounding land cover. The data upstream from the active gas well were compared to the data collected downstream from the well. The data were assessed to determine variation and whether or not variation that occurred was natural or due to other land use/cover factors between the upstream and downstream sites.

Two Mile Run is in Trout Unlimited's CSI immediate monitoring zone, indicating no current monitoring going on, but an immediate need for monitoring because of active gas wells in the area. The TU CSI monitoring zones are suggestions for the type of monitoring that should be conducted in those areas (Lemon, 2014). Zones are based on the locations of present shale gas wells, future shale gas development areas and the location of high quality wild and native trout populations (Fesenmyer, 2014). The well pad in the area is Range Resources' Carter R unit 4H unconventional, horizontal gas well is located southeast of sites 1, 2 and 3 (Figure 3). The well is within the Two Mile watershed (Figure 1), so runoff from the well pad is a potential threat to

the stream. Sites 1 and 2 are located upstream in the watershed from the location of the Range Resources active, unconventional, horizontal well pad (Figure 3).

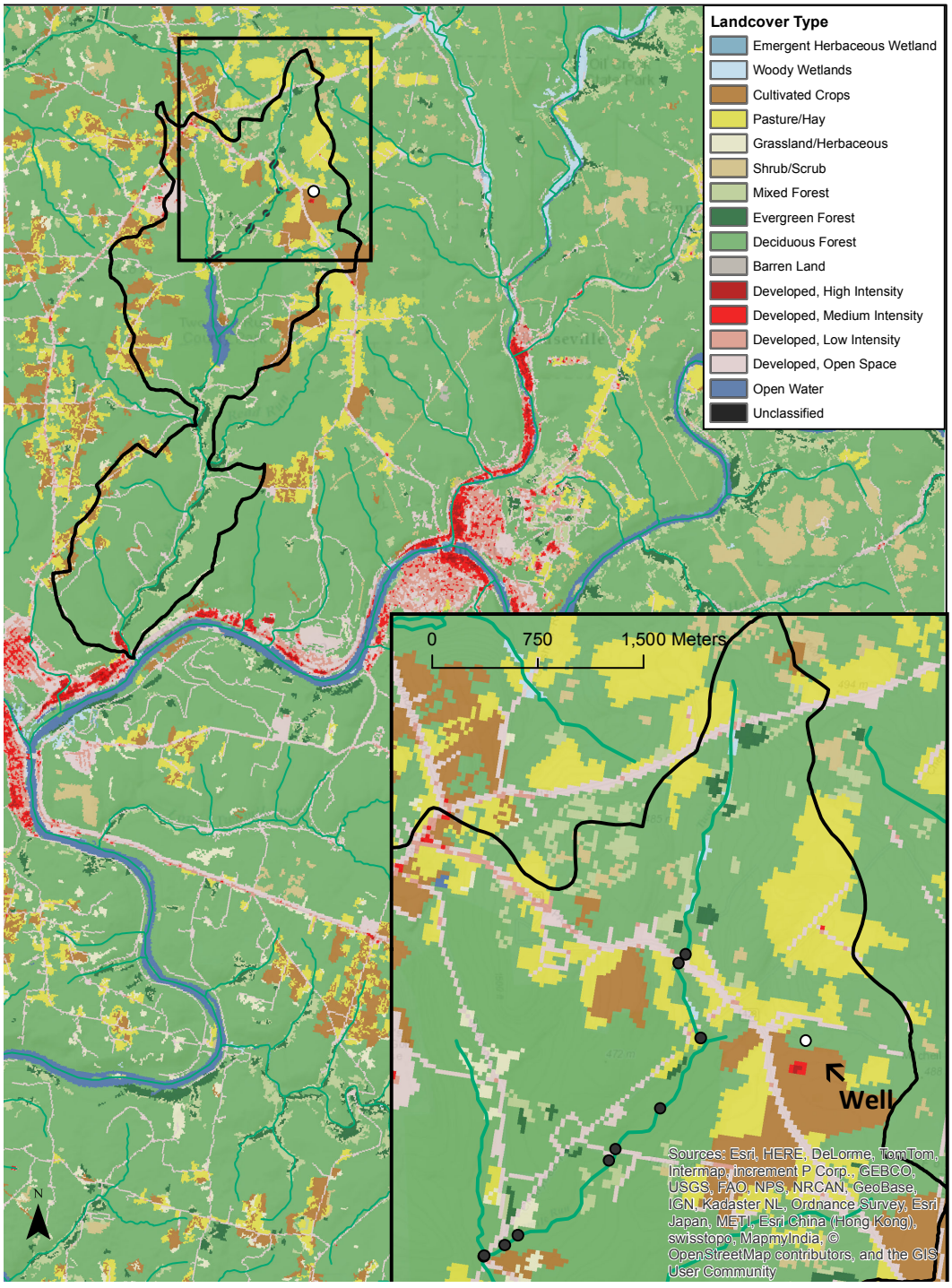


Figure 2. Land cover map of Two Mile Watershed, Venango Co., PA showing inset map of study area with locations of sample sites (black dots). The dominant land cover type around the stream and in the watershed is deciduous forest. Carter well pad is red space south of the white dot.

Sampling Methods Six sets of data were collected for each of the ten sample sites from October to December 2014. The samples were collected every two weeks beginning October 17, 2014 and ending December 23, 2014. The preceding tests were conducted in the field at each sample site:

Electrical Conductivity (EC), total dissolved solids (TDS), and water and air temperature were measured using a LaMotte Tracer PockeTester (Chestertown, Maryland) meter donated by Pennsylvania Trout Unlimited.

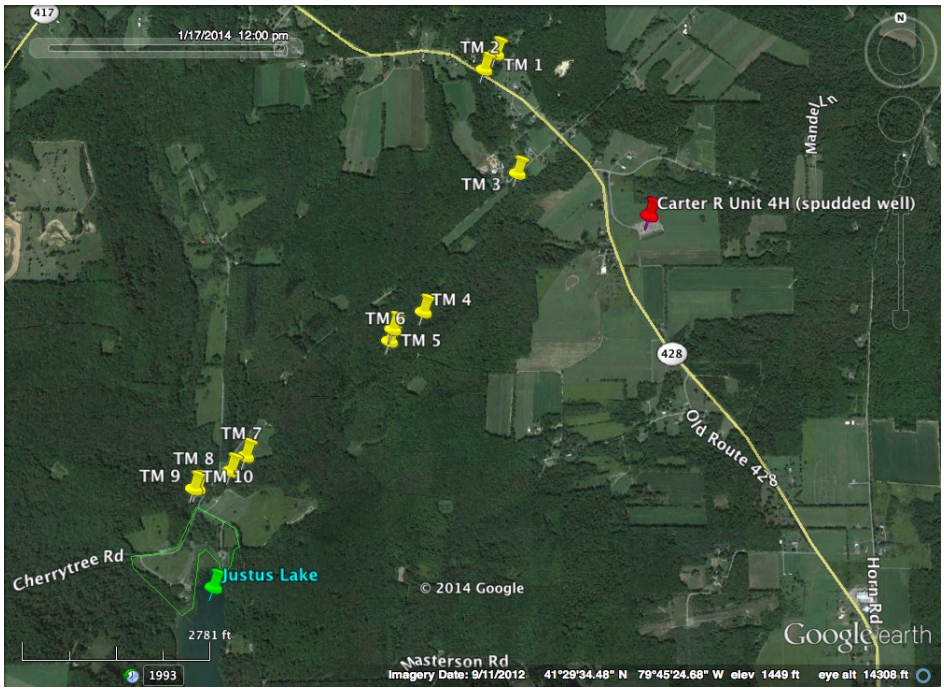


Figure 3. Aerial photograph of study area (copyright Google Earth). Sample site locations are in yellow, reservoir is in green and well pad is in red.

pH was measured using a Hanna pH Meter, pHep5 (Woonsocket, Rhode Island).

Ten depth measurements were taken across the width of the stream using a meter stick and average depth was calculated. The width of the stream was taken using measuring tape and cross-sectional area was then calculated (average depth multiplied by width).

Turbidity was measured by using Trout Unlimited's Lawrence Enterprises Inc. Secchi-tube (a turbidity tube measuring up to 120 centimeters with a small Secchi disk) (Louisville, Kentucky).

Many biotic indices show a substantial drop in taxa richness scores in the summer and early autumn, but show a rise in richness during the month of November (PA DEP, 2012). Therefore, diversity of benthic macroinvertebrates (BMIs) was sampled once in locations upstream and

downstream of the well, on November 23, 2014. BMIs were collected using a (0.5 mm size mesh) LaMotte kick net (Chestertown, Maryland) with a 1 x 1 meter square frame provided by Trout Unlimited. Samples were taken in riffle, run and pool habitats upstream and downstream from the well pad. Three, 20 second-kicks were done in each habitat type upstream from the well pad, and then the process was repeated downstream from the well pad. After kicking at one location, the net was taken out of the water and the sample was collected and preserved in 95% ethanol for later identification. The samples were divided into habitat type and upstream versus downstream samples and placed in corresponding containers. There were a total of six containers (three upstream riffle, run, pool, and three downstream riffle, run, pool containers). Samples were brought back into the lab and sorted. Due to time constraints, EPTs were identified to Family and all other specimens were identified to Order using a collection of taxonomic keys in Peckarsky et al. (1990) and Merritt et al. (2008) and only the riffle habitat samples were identified. EPTs are the most sensitive to physical and chemical change, so the diversity of those Orders were more important for assessing stream health than the other Orders. Riffle habitats have the highest taxa abundance and diversity because the flowing water mixes with the air and creates a more oxygenated zone than the other habitat types (Roy et al., 2003).

A subsampling method was used to identify macroinvertebrates. The method used in this study was a fixed count subsampling method based on protocol from Barbour and Gerritsen (1996). Each sample was added to a flat pan containing a grid of twelve squares. The pan was shaken and the specimens spread out so that the sample was evenly distributed on the grid. Five random squares from the grid were chosen to taken samples from (almost half of the total sample was identified). The fixed number of specimen picked from the subsample was set at 300, based on how many specimens were picked after the first subsample was taken. Each riffle habitat sample was estimated to be about the same size visually in abundance. Barbour and Gerritsen (1996) recommend that the number of specimens picked out of the sample must remain within the 20% of the targeted number (i.e. 240-360 per subsample) to prevent any error.

The current weather/precipitation was recorded as well as the precipitation from the last 48 hours. This was done using observation and local weather reports from the National Weather Service website (weather.gov).

A one-way ANOVA was performed to determine the significance of mean TDS, mean pH, mean electric conductivity, and mean water and air temperature (separately) in upstream sample sites compared to downstream sample sites. Another one-way ANOVA was performed for those same parameters comparing only the downstream sites. A Tukey Test was performed to determine the significance difference among downstream sample sites (Appendix A). Alpha was 0.05.

Rationale for Sampling Methods

Conductivity can give information about how the stream is fed (mostly groundwater or precipitation) and can give information about how the bedrock will withstand erosion. Streams with low conductivity tend to be precipitation fed and have bedrock made of materials that do not ionize easily with water (ex. granite bedrock) (U.S. EPA, 2012). According to the Alliance for Aquatic Resources Monitoring (2010), the range for conductivity in U.S. streams is 15-500 µS/cm.

A large increase in TDS indicates that water may be impacted by a spill or leaks from gas extraction or other land use activities. If the levels do spike, testing for Barium and Strontium III would be necessary in case the cause of the spike is a spill or leak of fracking fluid or flowback water, which contain high levels of salts and metals, from the well pad site. According to the Commonwealth of Pennsylvania Water Quality Standards (2009), the criteria for potable water supplies in Pennsylvania are an average of 500 mg/l and a maximum of 750 mg/l.

Many aquatic organisms are sensitive to changes to pH. Surface waters vary in pH because of materials flowing through them from the source, the composition of bedrock and surrounding soils and many other factors. The pH of healthy, natural waters in Pennsylvania ranges from 6.0 – 9.0 inclusive, which is a range in which most aquatic life can live (Commonwealth of PA, 2009).

The temperature of freshwater determines the rate of many chemical reactions and is a critical factor in the lives of aquatic organisms. Water temperature in PA streams change throughout the seasons. According to the Commonwealth of Pennsylvania Water Quality Standards (2009), the

average stream temperatures in October range from 10-12.22 degrees Celsius, 5.56-7.78 degrees Celsius in November, and an average of 4.45 degrees Celsius in December. These are critical temperatures for cold-water fisheries to survive.

Large amounts of silt and clay (soil erosion), algae (algal blooms from possible waste discharge or agricultural runoff, especially nitrogen and phosphorus fertilizers), organic and inorganic matter, or microorganisms can cause streams to have high turbidity. High turbidity can lead to an increase in water temperature because the suspended particles absorb heat more readily. Turbidity can also have an affect on macroinvertebrates and fishes that are visual predators or that are sensitive to changes in water temperature.

The abundance and diversity of BMIs is an important parameter in assessing stream health. BMIs are good indicators of stream health for a number of reasons. They are good indicators of local conditions because they generally stay in one place, they make up a broad range of species from different trophic levels and pollution tolerances, and they have short life cycles that are sensitive to stress (U.S. EPA, 2012). Certain orders of BMIs (Ephemeroptera, Plecoptera and Trichoptera) need high quality stream habitats to thrive. Some BMIs can survive in lower quality water conditions (Diptera, Tubificidae). BMIs that can survive in poor water quality habitats can be found in high quality water, but when they make up the majority of taxa found in a stream, it indicates poor stream health.

Using subsampling identification methods for macroinvertebrates is common in citizen science biomonitoring programs. However, as Baker and Huggins (2005) point out, many programs are employing subsampling methods without an analysis of the consequences of examining only a portion of the sample. Most of these subsampling methods are not standard, and this lack of research of the methods and lack of standardization could lead to useless information, waste of time and resources, or implementation of regulations based on inaccurate data. Two drawbacks to this method are that accurate total abundance data and richness per unit area cannot be determined. However, the later drawback is more troubling when comparing different streams. Because this study only focuses on one stream, and the locations were within a couple kilometers

of each other, this metric is not as troubling. Therefore, only taxa richness was able to be determined.

The cross-sectional area (the product of width and average depth) of the stream is another way to see how the stream is changing as each site and throughout the study time. It can also aid in determining whether the stream is being affected by excessive withdrawals or spills.

Other observations include land disturbances such as poor riparian zones and increased traffic on roads that are usually not used. Precipitation is important to record to help determine causes in an increase or decrease in stream flow.

Results

Overview

Sample sites 1 and 2 were located upstream from the Carter R unit 4H well in the Two Mile watershed and sample sites 3 through 10 were located downstream. The sampling dates are listed in Table 2.

Table 2. Dates that each set of samples were taken. A set is a day on which all ten samples were taken.

Set	Date
1	Oct. 17
2	Oct. 31
3	Nov. 12, Nov. 16
4	Nov. 21, Nov. 24
5	Dec. 7
6	Dec. 23

Abiotic Indicators

The mean TDS concentration was statistically significant between upstream and downstream sites ($p=0.0062$; $p<0.05$: $F=8.0656$). On November 21 and 24 (set 4), mean TDS increased in the downstream sample sites (Figure 4). The mean TDS concentration was not statistically significant among downstream sample sites ($p=0.8533$; $p<0.05$; $F=0.4659$).

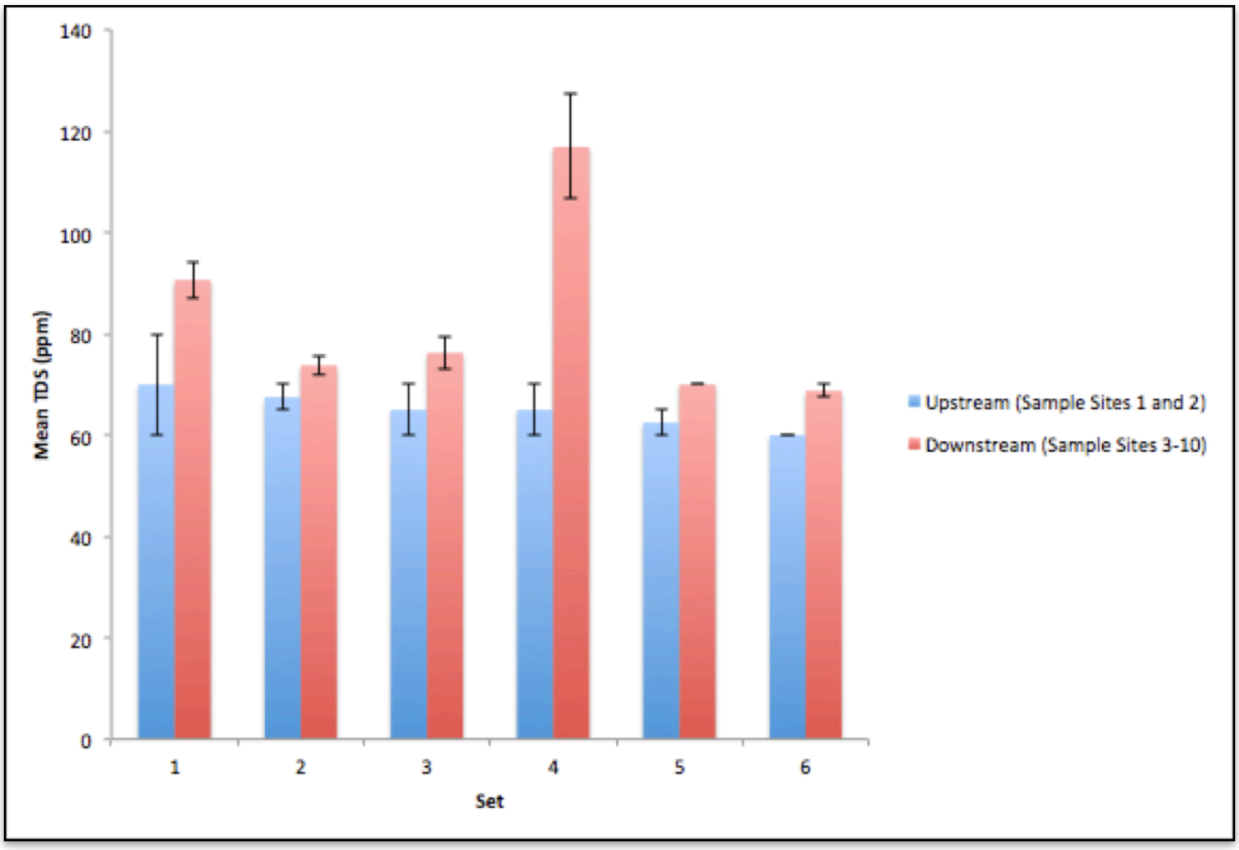


Figure 4. Average TDS concentration in sites upstream versus downstream of the Carter well for each sampling date (set). This shows an increase of TDS in downstream sites vs. upstream sites throughout study period. The dates for sets 1-6 can be found in Table 2.

Mean conductivity was statistically significant between upstream and downstream sites ($p=0.0043$; $p<0.05$; $F=8.8399$). Conductivity values were higher in the downstream sites than in the upstream sites. There was a large increase in conductivity values in set 4 in the downstream sites (Figure 5). Mean conductivity was not statistically significant among downstream sample sites ($p=0.8743$; $p<0.05$; $F=0.4350$)

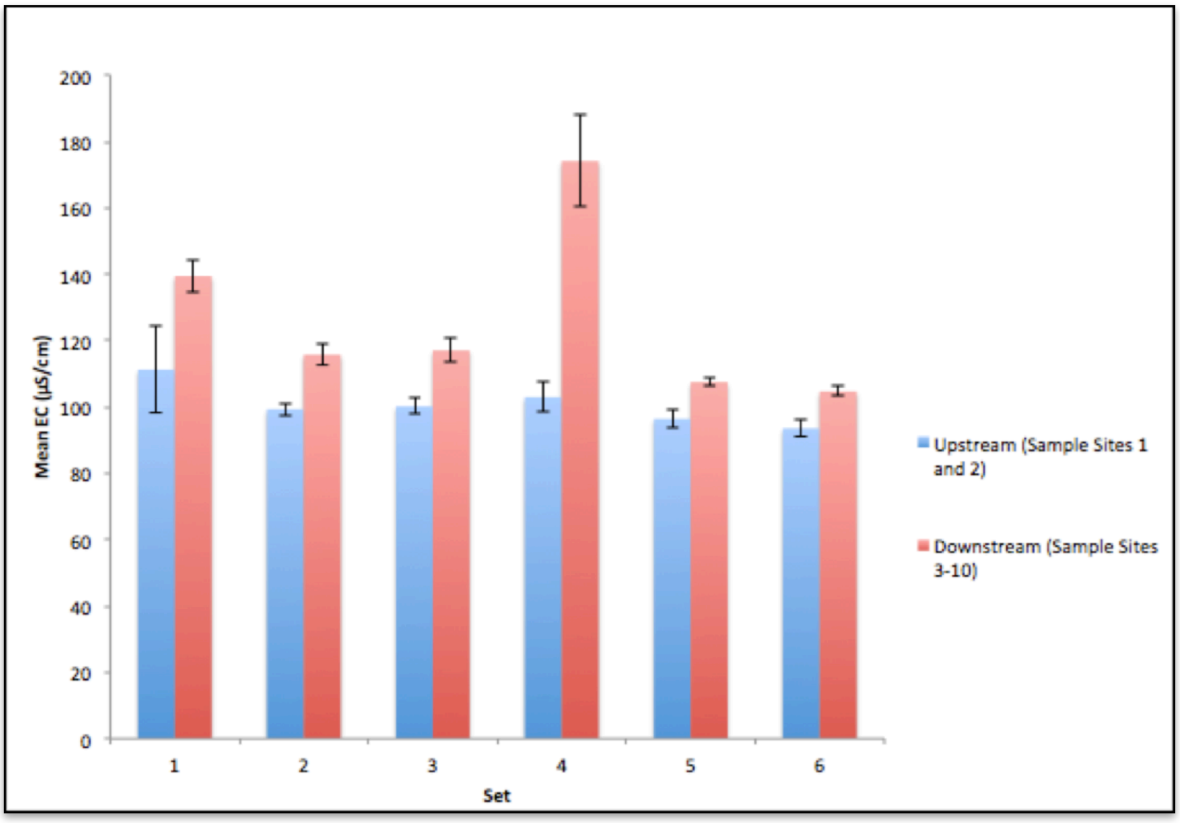


Figure 5. Average conductivity in sites upstream versus downstream of the Carter well for each sampling date. Shows increase of conductivity in downstream sites vs. upstream sites throughout study period. The dates for sets 1-6 can be found in Table 2.

Mean pH was statistically significant between upstream and downstream sites ($p=0.0001$; $p<0.05$; $F=16.5436$) (Figure 6). The pH gradually increased along the length of the study area from site 1 to 10 (Appendix A, Figure 12). Mean pH was statistically significant among downstream sample sites ($p=0.0001$; $p<0.05$; $F=5.7109$). The pH in the downstream sites was higher than the pH of the upstream sites. The pH at any point in the study area was still within the average range for PA streams, which ranges from 6.0 to 9.0 (Commonwealth of PA, 2009).

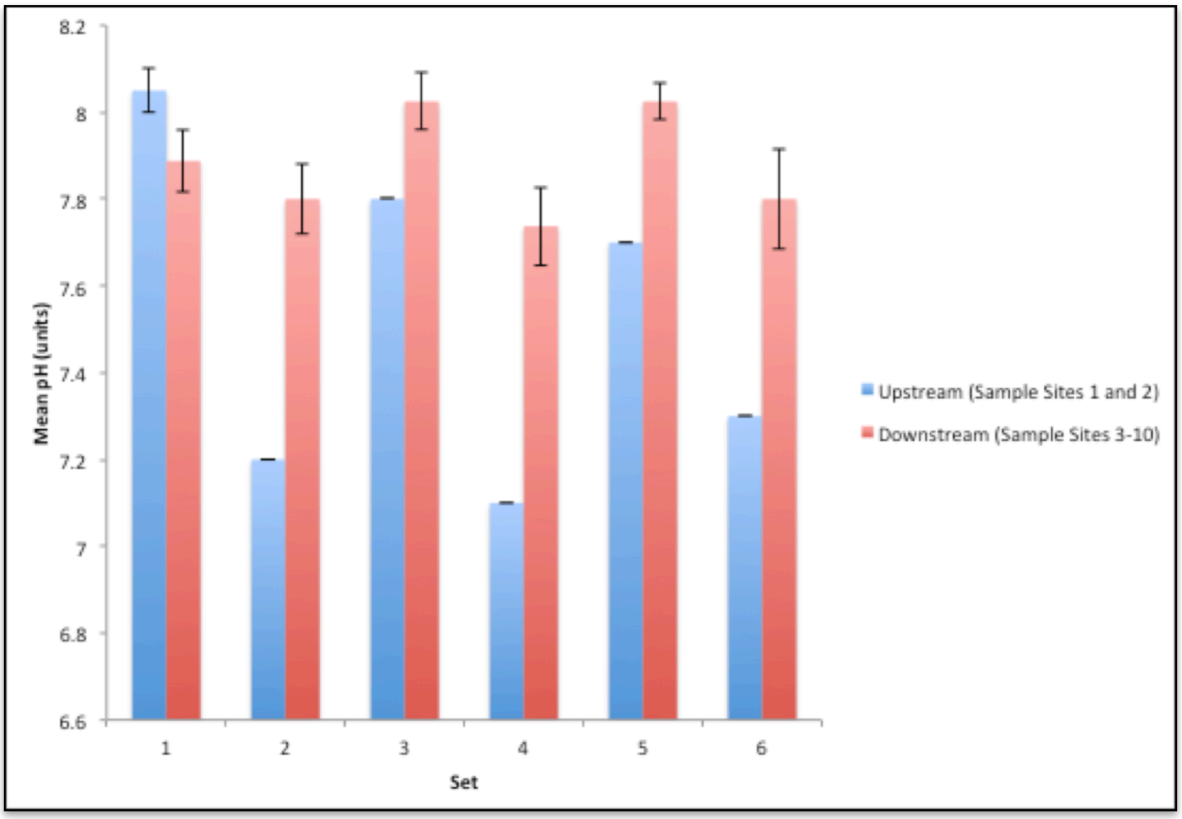


Figure 6. Average pH in sites upstream versus downstream of the Carter well for each sampling date. Shows increase of pH in downstream sites vs. upstream sites throughout study period. The dates for sets 1-6 can be found in Table 2.

Mean water temperature was not statistically significant between upstream and downstream sites ($p=0.9187$; $p<0.05$; $F=0.0105$). The water temperature kept fairly consistent throughout the entire study area. Sets 1 and 2, taken in October, were within the average PA water temperature for coldwater fisheries range of 10-12.2 degrees Celsius. Sets 3 and 4, taken in November, were slightly colder than the average PA water temperature range of 5.6-7.8 degrees Celsius. Sets 5 and 6, taken in December, were within the PA water temperature average of 4.5 degrees Celsius. Mean water temperature was not statistically significant among downstream sample sites ($p=0.9998$; $p<0.05$; $F=0.0488$). The water temperature remained fairly consistent throughout the downstream sites. There were greater water temperature changes between sites in sets 3 and 4 (Appendix B and C).

The mean air temperature fluctuated slightly throughout each day during which the samples were taken but was not statistically significant between upstream and downstream sites ($p=0.7103$;

p<0.05; F=0.1394). Mean air temperature was not statistically significant among downstream sample sites (p=0.9966; p<0.05; F=0.1193). There were greater water temperature changes between sites in sets 3 and 4 (Appendix B and C).

Turbidity was not analyzed using statistics. Observations were made, and the Secchi disk was visible for 120 centimeters or more in the upstream sample sites more often than in the downstream sites for every set. The water was more turbid during set 4 both upstream and downstream, although the water in the upstream sites was still clearer than the downstream sites on average (Appendix B and C).

Table 3. Relative diversity of macroinvertebrate Orders and Families. X's represent the presence of a group of taxa in upstream or downstream riffle habitats. The PA DEP (n.d.) taxa pollution tolerance index is provided as well (0 = intolerant, 10 = tolerant). The percent of each Family or Order found out of the total subsample for upstream and downstream sites.

Order	Family	Upstream	% Found in Sub-sample (upstream)	Downstream	% Found in Sub-sample (downstream)	DEP Taxa Tolerance Index
Ephemeroptera	Heptageniidae	X	6.85	X	10.89	3
	Leptophlebiidae	X	2.42	X	9.37	4
	Ephemerellidae	X	0.81	X	8.10	2
	Baetidae		0	X	3.04	6
Plecoptera	Perlidae	X	0.40	X	2.53	3
	Perlodidae	X	18.95	X	10.89	2
	Chloroperlidae	X	6.05	X	5.82	0
	Capniidae		0	X	0.76	3
	Taeniopterygidae		0	X	5.57	2
	Unidentified	X	9.27	X	4.56	N/A
Trichoptera	Hydropsychidae	X	3.23	X	6.84	5
	Philopotamidae	X	0.81	X	2.53	3
	Limnephilidae	X	2.02		0	4
	Uenoidae		0	X	2.78	3
	Brachycentridae		0	X	0.76	1
	Polycentropodidae		0	X	1.27	6
Coleoptera	N/A	X	2.42	X	3.80	N/A
Diptera	N/A	X	45.56	X	19.24	N/A
Megaloptera	N/A	X	1.21		0	8
Annelida	N/A		0	X	0.76	N/A
***Phylum Mollusca, Class Bivalvia**	N/A		0	X	0.51	N/A

Macroinvertebrate Assemblages

An estimate of relative diversity was determined (Table 3). Multiple Families of EPTs were present in both upstream and downstream samples. Coleoptera, an overall stream diversity indicator Order was present in both upstream and downstream samples as well.

Discussion

Abiotic Indicators

There was an increase in TDS concentration in the downstream compared to upstream sites. Set 4 shows the highest TDS concentrations. The concentrations were within the average range for TDS in PA streams, which is an average of 500 ppm and a maximum concentration of 750 ppm (Commonwealth of PA, 2009). However, the increase was statistically significant between upstream and downstream sites, which is over a couple thousand meters (relatively short distance), indicating something going on that is not naturally occurring. The major increase in TDS during set 4 was most likely due to snowmelt occurring from the warmer temperatures after snowfall on the previous days.

The gradual increase in conductivity values, which mimicked the trend of TDS concentration, was also within the average range for conductivity in U.S. streams, which is a range of 15-500 μS/cm (ALLARM, 2010). However, the increase was also statistically significant between upstream and downstream sites, over a short distance, indicating something going on that is not naturally occurring. Set 4 also showed the highest conductivity values downstream compared to the other sets. This increase was also most likely due to snowmelt occurring from the warmer temperatures after snowfall on the previous days.

As mentioned in the introduction section of this study, TDS and conductivity can increase due to sedimentation or other chemicals entering the stream through many different pathways such as tributaries, infiltration, or runoff from fields and roads (Allan and Castillo, 2007). Transportation of materials to and from the well pad on dirt roads is one specific pathway that sediments can enter tributaries. Other pathways that chemicals, salts and other particles from the well pad enter the stream include runoff from retention ponds or leaks in well casing entering groundwater (Ingraffea, 2014).

The pH values along the stream were all within the natural pH values for Pennsylvania, however, there was a significant difference between upstream and downstream sites, indicating some major change occurring along the stream. The pH was increasing as the water flowed downstream. Although pH naturally increases in all streams, the significant increase indicates some impact on the stream, potentially from activity at the well combined with the pH buffering effects of the limestone bedrock found in the stream.

The temperature of the water remained generally constant throughout the sites. As the seasons changed, the air and water temperatures changed as well. The water temperature remained within the average ranges of PA streams, however sets 3 and 4, taken in November, had slightly colder temperatures than the average of 5.6-7.8 degrees Celsius (Commonwealth of PA, 2009). The unseasonably colder air temperatures and snowmelt can explain this change during that period of time.

Statistics were not analyzed for turbidity data, however, by observing Figure 9, one can see that the results show no general increase in turbidity measurement seasonally. Figure 15 shows a site-to-site comparison of turbidity measurements. According to the results in this study, turbidity generally remained in a range of 113-120+ cm visibility. Set 4 indicates higher turbidity (80-100 cm visibility), which can be explained by the snowmelt event mentioned previously.

TDS and conductivity results show a general decrease in measurements overtime (seasonally) with the exception of set 4 (Figures 4 and 5, respectively). The measurements generally decrease from October to December, which is consistent with PA natural water quality standards, however the TDS for each set, regardless of seasonality, was significantly different in upstream versus downstream sites.

The land cover surrounding the streams was mostly composed of deciduous forests with some mixed forests, evergreen forests and hay/pasture land. The majority of the sites were in a county park used mainly for recreational uses such as hunting, fishing, hiking and biking. Because the stream had forested riparian zones, runoff from the hay/pasture land and compact dirt and paved

roads did not, and would not be expected to, affect the stream reach in major ways. The Range Resources shale gas well is the only active natural resource extraction operation going on in the study region of the watershed that would affect the reach. This operation is the only major potential risk to the integrity of Two Mile Run.

Macroinvertebrate Assemblages

The presence of extremely sensitive taxa (EPTs) in both upstream and downstream sites indicates high water quality conditions above and below the well. The abiotic factors also indicated high water quality; meaning macroinvertebrate diversity would not be expected to significantly change from the upstream to the downstream sites (Allan and Castillo, 2007). The subsampling results showed that there were more Families of EPTs present in downstream sites compared to the upstream sites. Reasons for this could include riffle habitats in the upstream sites were less abundant and of poorer quality (did not have as many white caps, less oxygenated) than the downstream riffle sites. The upstream macroinvertebrate samples were taken at the end of the day when there was less daylight, so some macroinvertebrates may have been left on the kick net due to poor visual conditions. The fixed-count subsampling method could not accurately calculate total taxa abundance, which, combined with taxa richness/diversity, would have given more information about the macroinvertebrate community in Two Mile Run (Barbour and Gerritsen, 1996). However, the fixed-count method provides standardization and saves time. The presence of multiple sensitive Families above and below the well is enough to show that the stream is currently healthy enough for these sensitive taxa to live. Total abundance would have to be determined in order to assess whether or not these taxa are thriving in one part of the stream or the other.

Limitations

The location of the Carter well made it difficult to find upstream sample site locations. The well was near the top of the watershed and the beginning of Two Mile was not too much farther upstream from that location (see Figure 1 for reference). That area was heavily forested private property, which also deterred me from locating more sample sites in the upstream region of the stream. The flow meter used to measure stream velocity was not available for the first two sets of data and the battery unexpectedly died in the field during the last set of data.

Conclusion

The objectives of this study were to assess water quality parameters to detect variability along Two Mile Run and determine whether any variability found was due to natural stream processes, shale gas well, or other land cover/land use cause. The hypotheses for this study were (1) water chemistry parameters (TDS, turbidity, conductivity and pH) would increase in downstream sites and (2) macroinvertebrate diversity would show worsened stream quality downstream. The results of this study show that there is an increase in water chemistry parameters from sample site 1 (upstream) to site 10 (downstream). The increase in TDS, conductivity and pH were significant between upstream and downstream sites. The significant increase indicates something more than just natural bioaccumulation is occurring because the study stretch of the stream is only 3,000 meters (a short distance for such significant results. There were some instances when the measurements increased more drastically than the others, but those instances were explained by weather events and still remained in the natural ranges of each parameter for the region. The macroinvertebrate assemblages showed more diversity downstream from the well, however, extremely sensitive taxa were found in both upstream and downstream sites, indicating that Two Mile Run has macroinvertebrate diversity of EPTs. The consequences of variations in parameters that are close to the averages, such as the results of this study, do not appear to affect the biological community drastically. However, if the results showed numbers for parameters that deviated from the ranges and averages for the region, extremes, chemical and physical variation can significantly affect the distribution of organisms (Allan and Castillo, 2007).

It is important to note that continued activity at the Carter well site poses potential risks for impacts on Two Mile Run and surrounding tributaries. Biomonitoring of this sub-watershed and other sub-watersheds with gas wells is important in order to protect the integrity of the individual streams as well as bigger, surrounding watersheds. This study can be used as an example of simple biomonitoring practices that can be employed to assess changes in headwater streams at risk for potential anthropogenic impacts. The study is also a reminder that there are natural changes in stream chemistry and biota from headwater to river, and no major changes in water quality parameters is good news. Data from this study helps show that although there is some effect going on in the stream, it is not extreme enough to cause major changes in biological

distribution. This study and other studies that have not found significant land use impacts to streams are still very useful for providing foundational data
for future studies.

D'Amico's study also provides foundational data on this stream. The results of the study showed Two Mile Run having the greatest change in pH upstream and downstream (7.8 - 8.3, respectively) and highest average turbidity in upstream and downstream sites compared to the other sites that were sampled (D'Amico, 2013). This study was done two years ago duration the construction of the Carter well pad. The results in 2012-2013 are consistent with the results of this study. This correlation indicates that there has been no significant change in parameters in the past two years, since extraction has begun.

In the future, biomonitoring should continue on this stream because it is a headwater stream to the Allegheny River and is within the sub-watershed of a shale gas well. There are other active shale gas wells in Venango and surrounding counties that have the potential to impact surrounding watersheds. Non-state or federal agencies are the best options for cheap and effective biomonitoring efforts. They should be employed to conduct volunteer biomonitoring programs in order to develop a collection of data for the area so changes in stream quality can be assessed throughout the active operation of the wells as well as any effects of abandoned wells. As hydraulic fracturing continues to become this country's major natural gas extraction method, biomonitoring programs will become more necessary to provide accountability for oil and gas companies as well as protect this country's precious streams and the organisms living in them.

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Appendix A: Column charts of parameter results with Tukey Test scores for downstream sites.

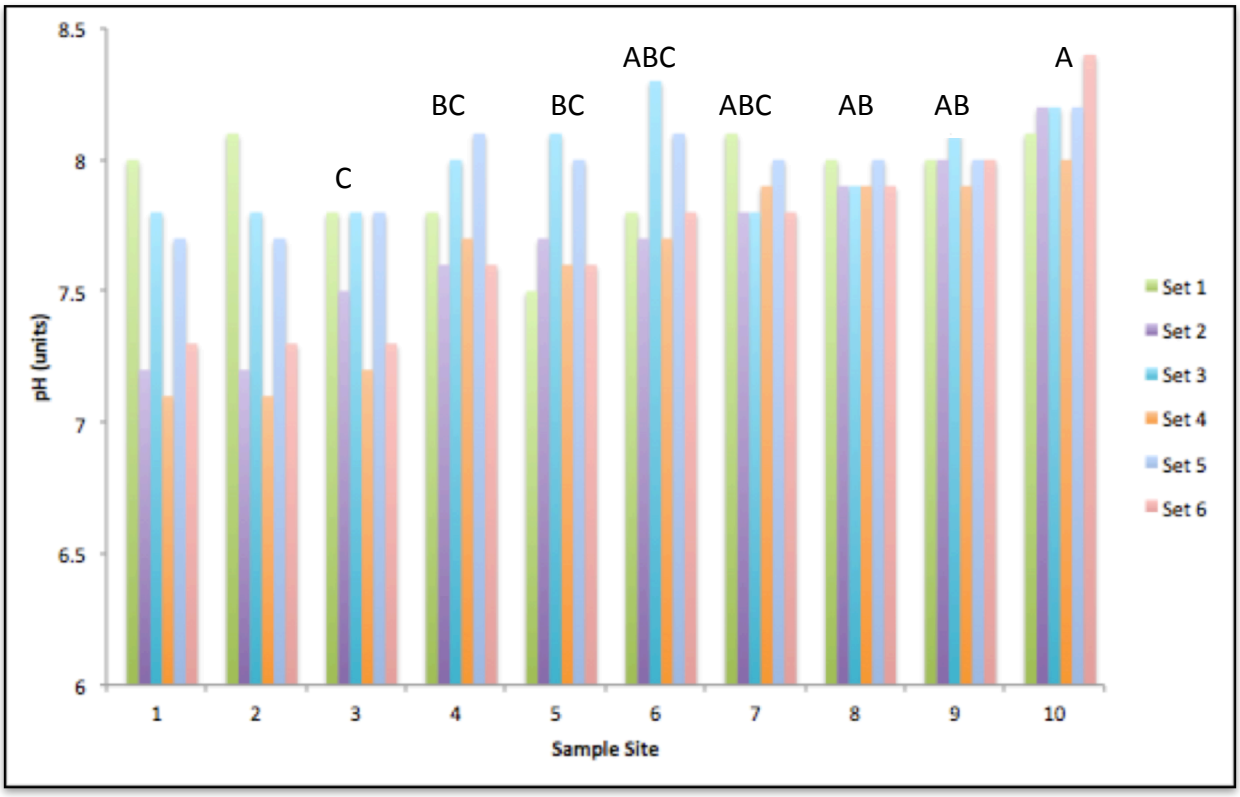


Figure 7. Chart of pH values for each sample site for each set. The dates for each set are in Table 2. This chart shows an increase in pH at each site moving downstream. This chart also displays a comparison among the sets (seasonality). The letters above the bars indicate the results of the Tukey Test. Sites with the same letter are statistically similar.

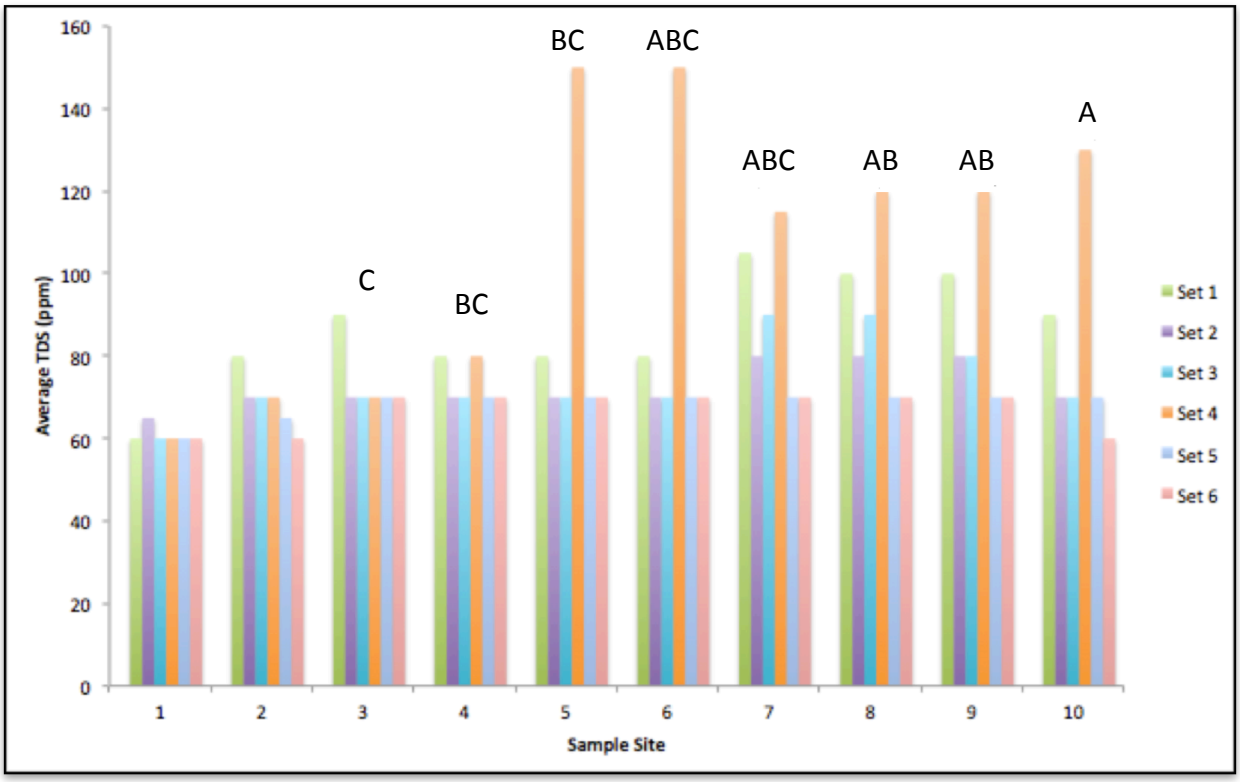


Figure 8. Chart of TDS concentration for each sample site for each set. The dates for each set are in Table 2. This chart shows a stable TDS concentration along the course of the stream reach. This chart also displays a comparison among the sets (seasonality). The effects of the snowmelt event in set 4 are evidence in this chart. The letters above the bars indicate the results of the Tukey Test. Sites with the same letter are statistically similar.

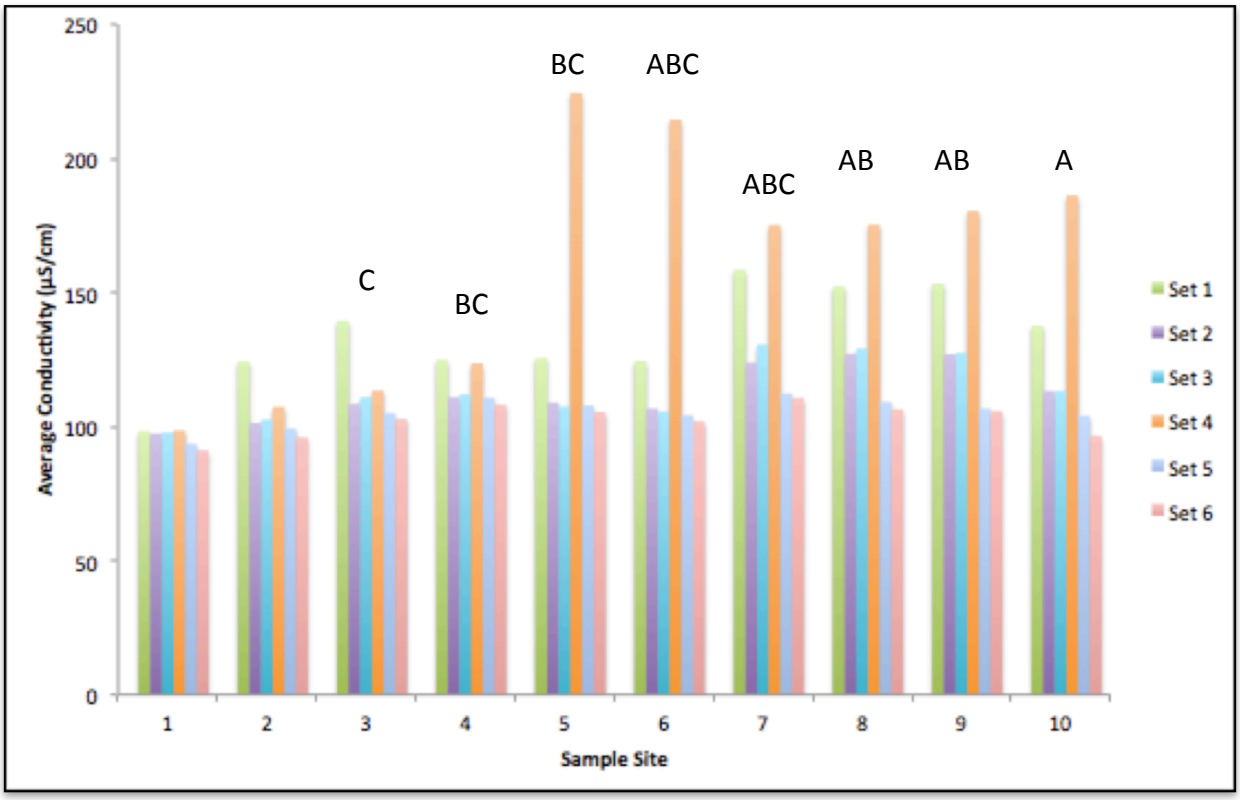


Figure 9. Chart of conductivity values for each sample site for each set. The dates for each set are in Table 2. This chart shows a stable conductivity along the course of the stream reach. This chart also displays a comparison among the sets (seasonality). The effects of the snowmelt event in set 4 are evidence in this chart. The letters above the bars indicate the results of the Tukey Test. Sites with the same letter are statistically similar.

Appendix B: Supplemental column charts displaying comparison of upstream versus downstream sites for other parameters not mentioned in the Results section of this paper.

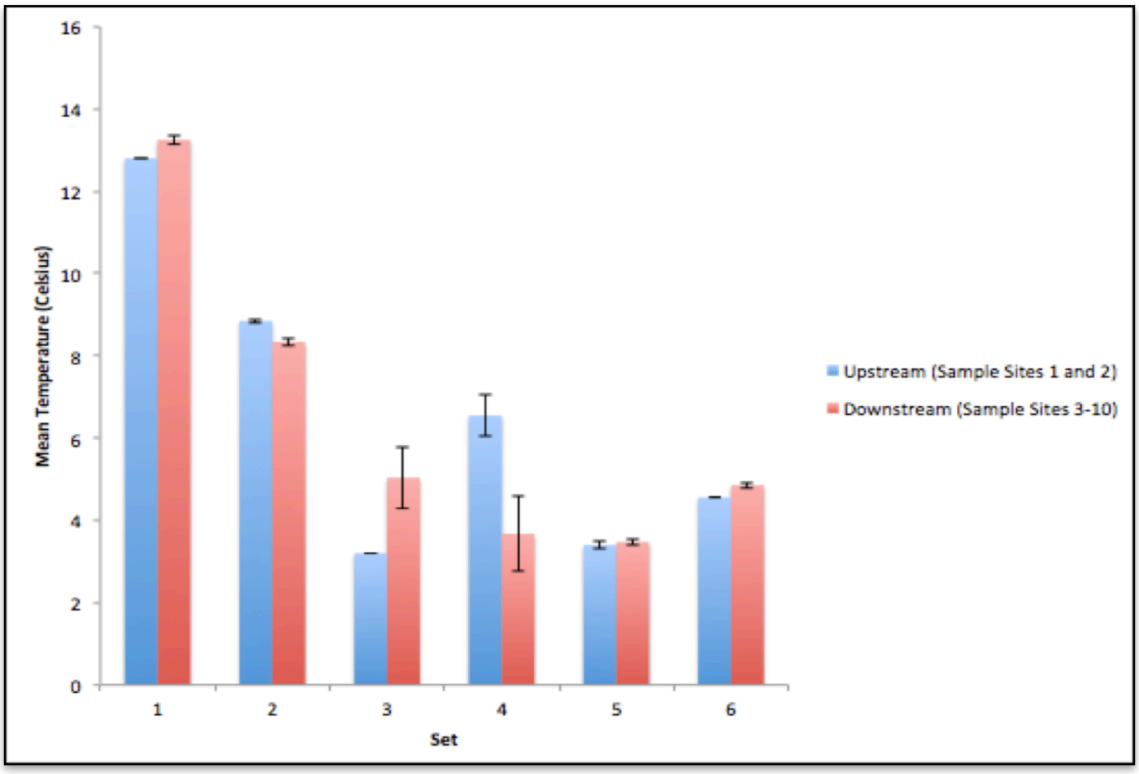


Figure 10. Chart showing the mean water temperature in upstream sites vs. downstream sites for each set. The dates for each set are in Table 2. The water temperature was not significant in upstream sites compared to downstream sites. A general decrease in overall water temperature can be seen as the seasonal temperatures decrease.

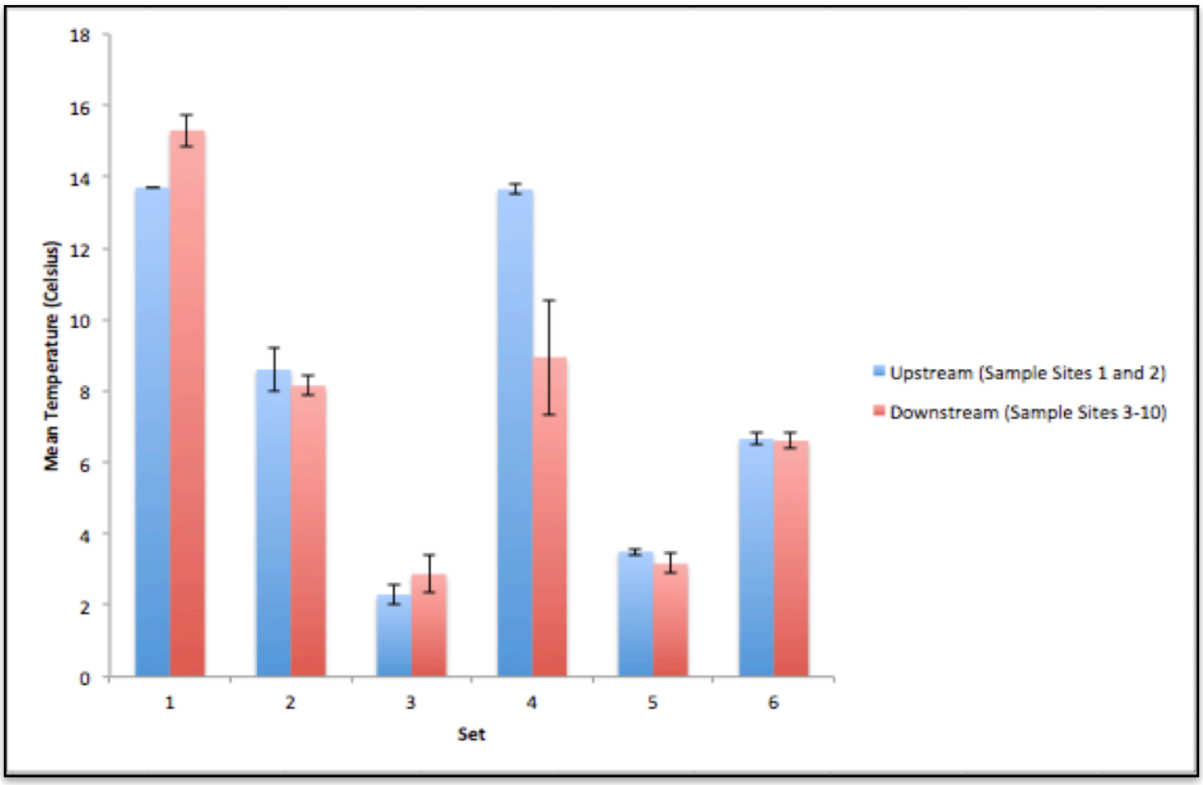


Figure 11. Chart showing the mean air temperature in upstream sites vs. downstream sites for each set. The dates for each set are in Table 2. The air temperature was not significant in upstream sites compared to downstream sites. A general decrease in overall air temperature can be seen as the seasonal temperatures decrease.

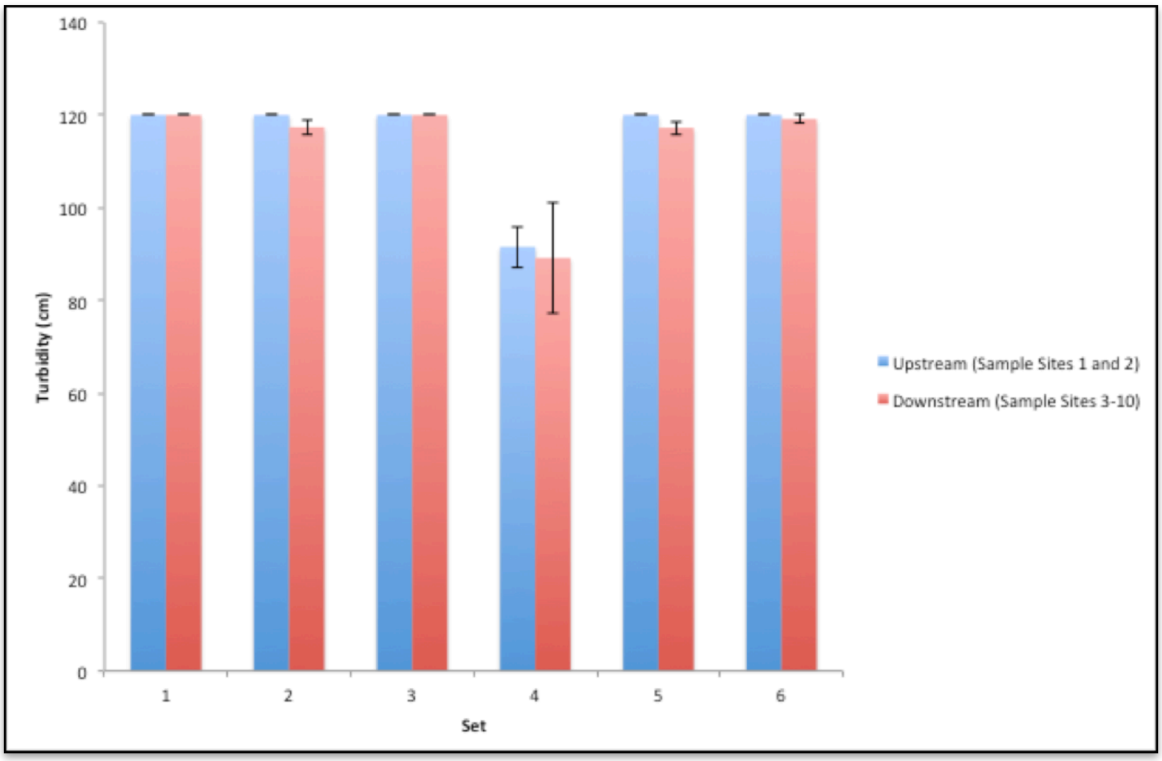


Figure 12. Chart showing turbidity values in upstream sites vs. downstream sites for each set. The dates for each set are in Table 2. Turbidity was not significant in upstream sites compared to downstream sites. Set 4 shows the snow melt event which lead to higher turbidity values in all sample sites.

Appendix C: Supplemental scatter plots displaying slopes of the changes in each parameter along the study stream reach for each set.

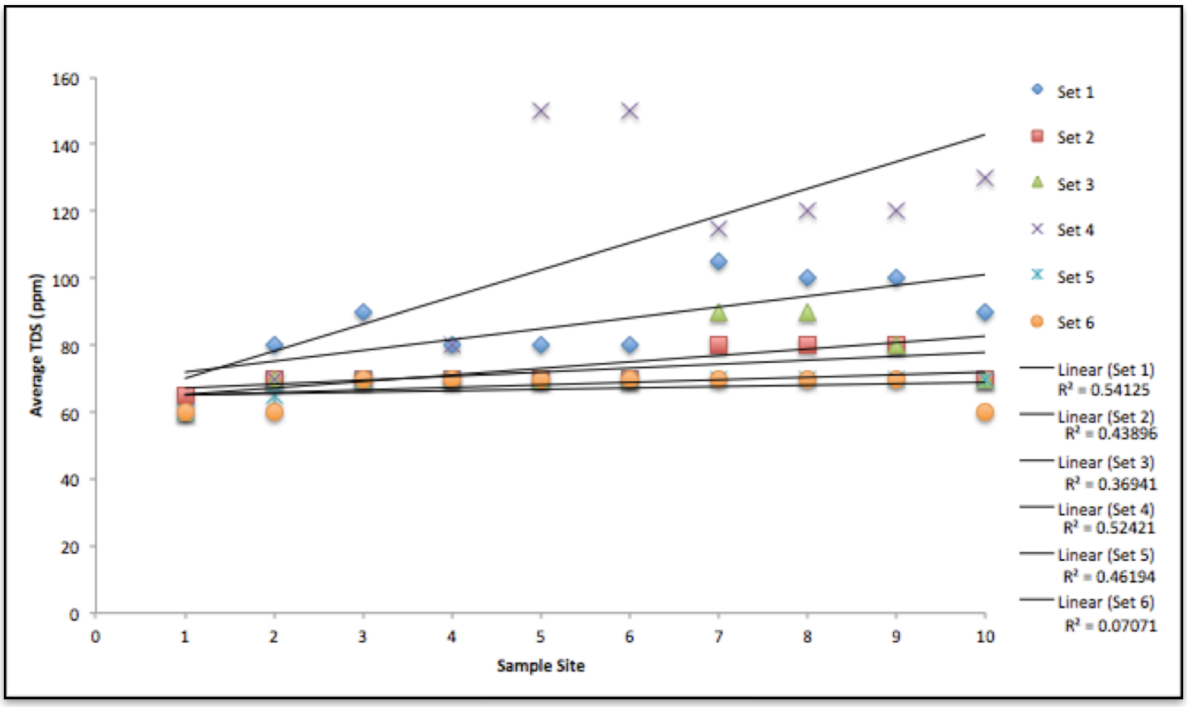


Figure 13. Chart showing the gradual change in mean TDS along the stream reach for each set. The dates for each set are in Table 2. A general increase in TDS concentration can be seen for each set.

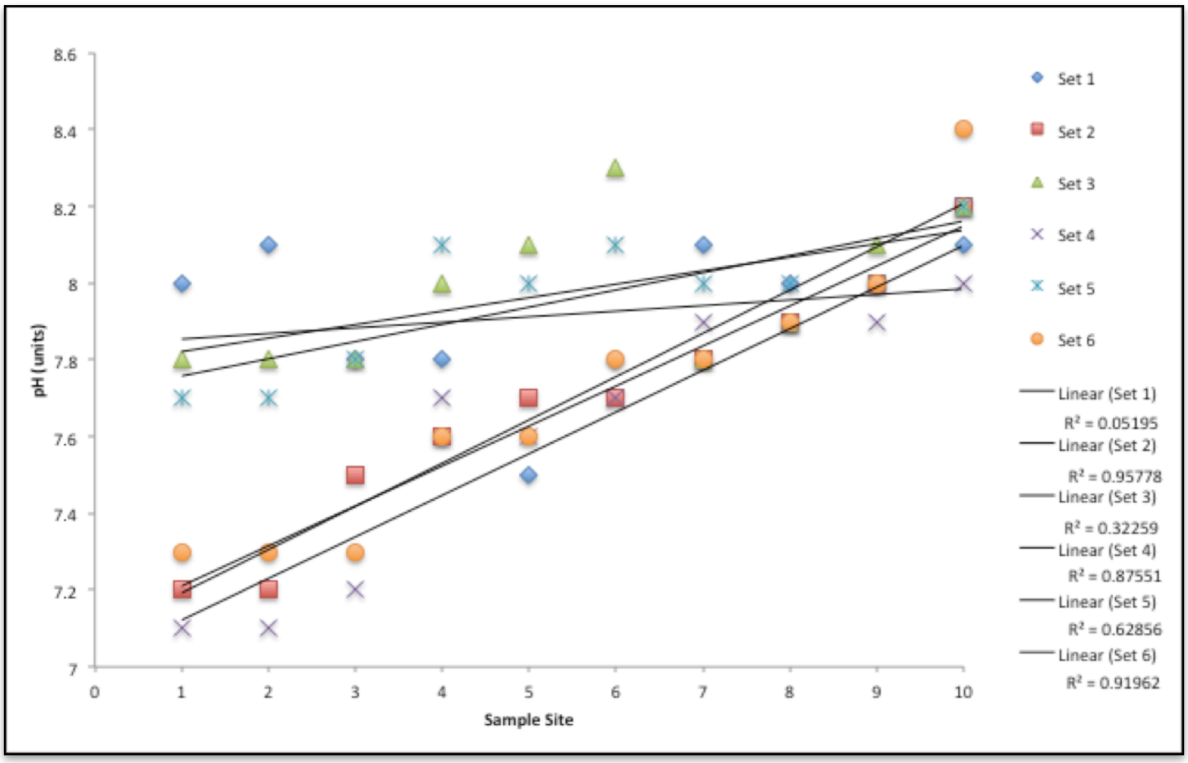


Figure 14. Chart showing the gradual change in pH along the stream reach for each set. The dates for each set are in Table 2. A general increase in pH values can be seen for each set from sites 1-10.

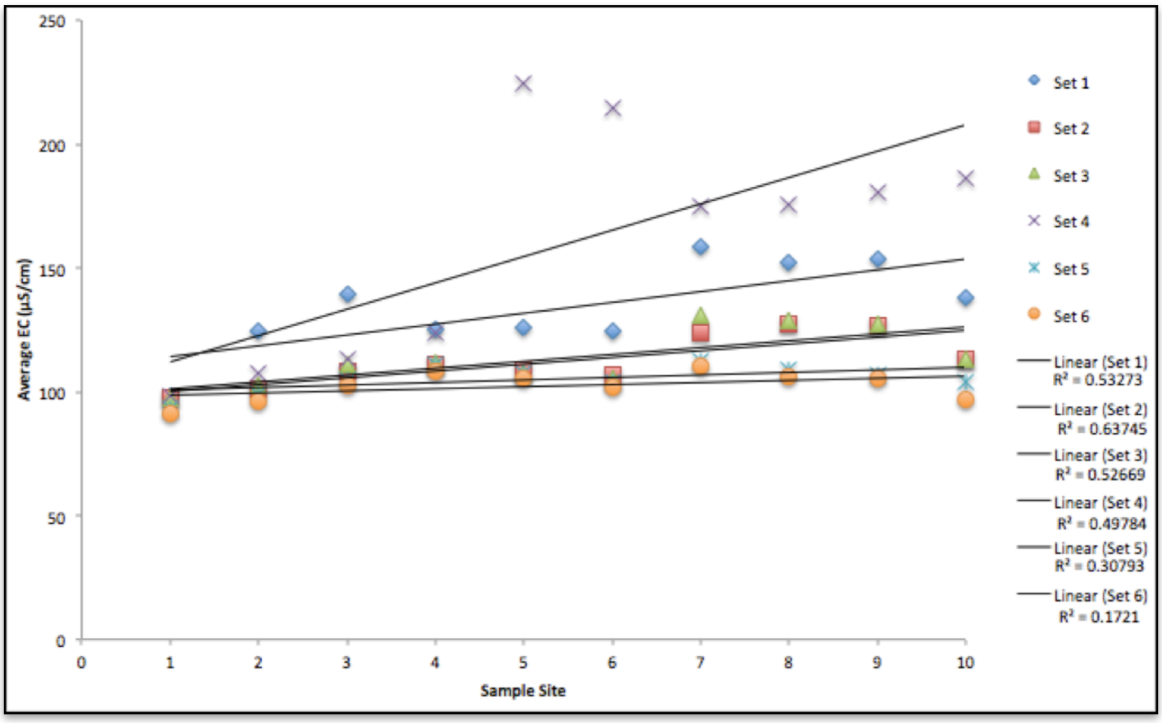


Figure 15. Chart showing the gradual change in mean conductivity along the stream reach for each set. The dates for each set are in Table 2. A general increase in conductivity values can be seen for each set.

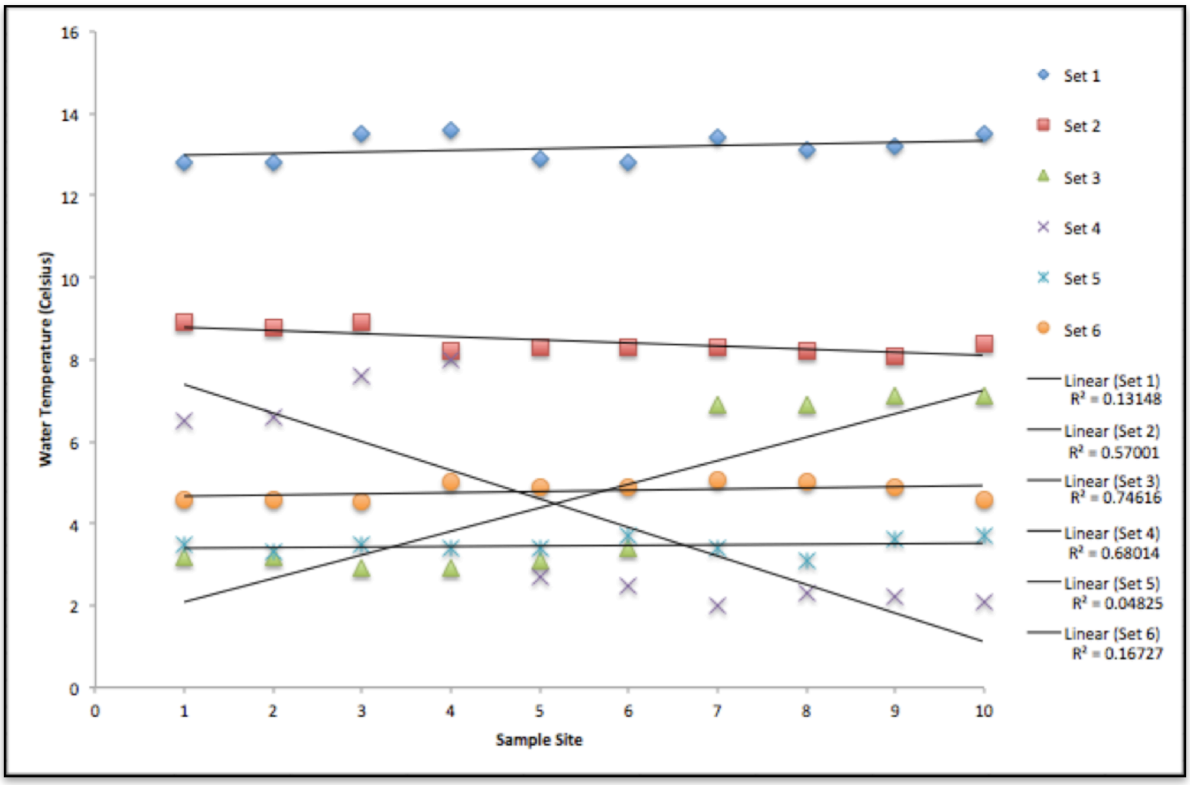


Figure 16. Chart showing the water temperature along the stream reach for each set. The dates for each set are in Table 2. Water temperature was not significant between upstream and downstream sites and generally remained consistent throughout the stream reach.

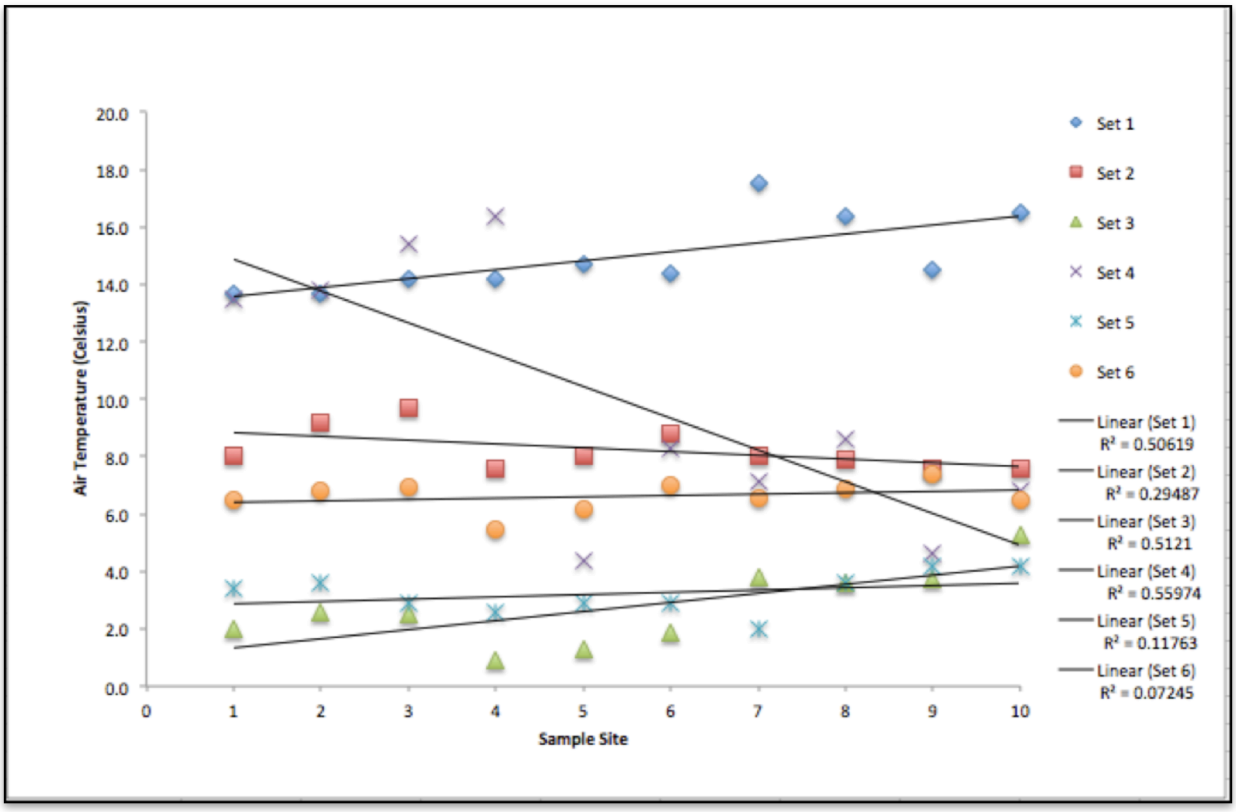


Figure 17. Chart showing the air temperature along the stream reach for each set. The dates for each set are in Table 2. Air temperature was not significant between upstream and downstream sites and generally remained consistent throughout the stream reach.

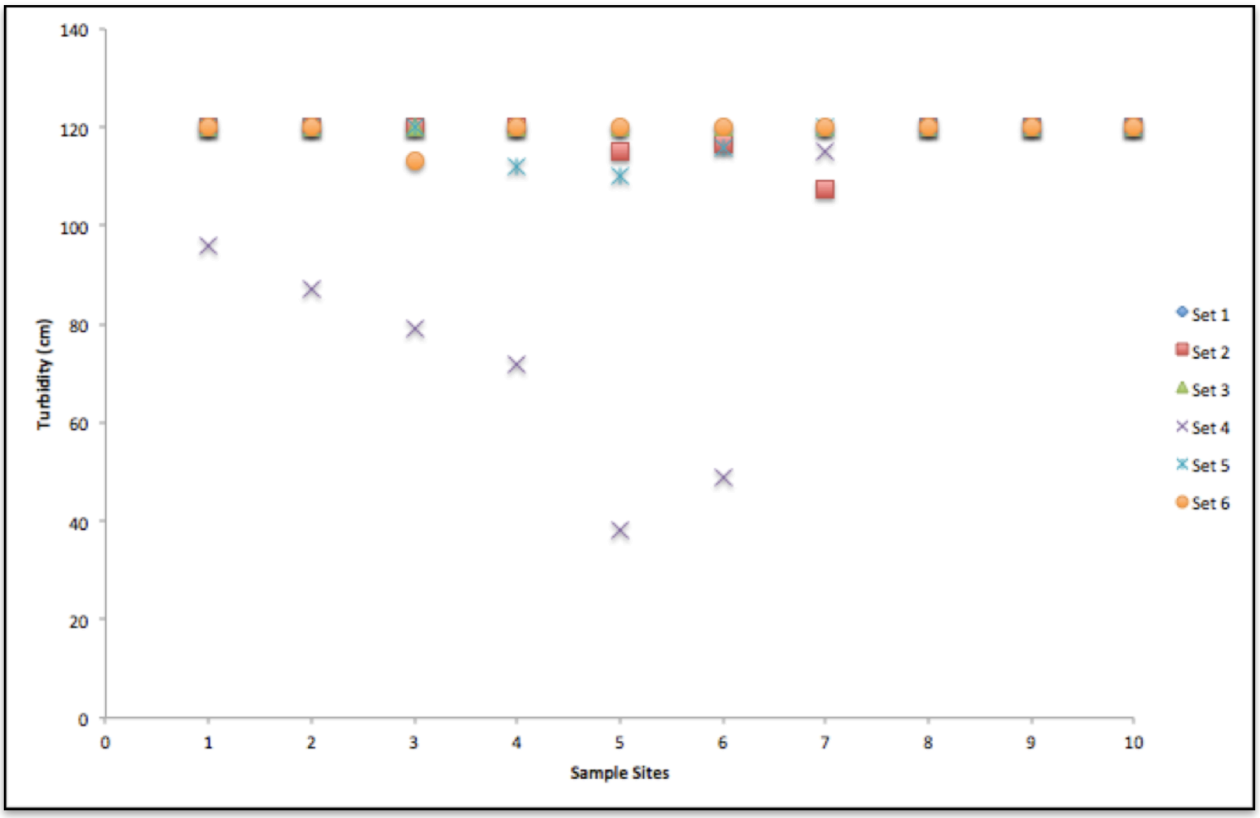


Figure 18. Chart showing turbidity along the stream reach for each set. The dates for each set are in Table 2. Turbidity was not significant in upstream sites compared to downstream sites. Set 4 shows the snow melt event which lead to higher turbidity (lower visibility) values.