Three Fork Creek Watershed Restoration
Status Report
Biological Monitoring Results

Prepared for:

WV Department of Environmental Protection
Division of Land Restoration
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February 6, 2020

Table of Contents

Table of Figures

List of Tables

1.0 Introduction

In August of 2009, the Watershed Assessment Branch (WAB) of the West Virginia Department of Environmental Protection (WV DEP) initiated a study to assess and monitor the biological health of Three Fork Creek. For many decades, acid mine drainage (AMD) from pre-law mining activities has negatively impacted the water quality of this watershed. This study is part of a collaborative effort with the Office of Abandoned Mine Lands and Reclamation (AML) of WVDEP and the West Virginia Division of Natural Resources (WVDNR). The primary objective of this collaboration is to improve water quality on several miles of Three Fork Creek. Expected benefits of improving water quality in Three Fork Creek include restoration of the benthic macroinvertebrate community and the establishment of a diverse fishery.

To improve water quality on the mainstem of Three Fork Creek, the AML program has successfully installed four treatment stations that release alkaline material into streams that neutralize the effects of AMD. These treatment stations have been in place and operating since the Spring of 2011. Personnel from both AML and WAB have been monitoring the watershed at several locations in an effort to determine how successful the current levels of treatment are at improving water quality in Three Fork Creek. This status report focuses primarily on Three Fork Creek mainstem and presents results of biological surveys conducted by WAB over the past 10 years involving the collection of fish and benthic macroinvertebrates, with an emphasis on comparing pre- and post-treatment data. Habitat and water quality information is also discussed.

2.0 Watershed Description

Three Fork Creek is located in Taylor, Preston, and Monongalia counties in northern West Virginia (Appendix B- Figure 21). The watershed drains approximately 101 square miles (65,920 ac). Watershed elevations range from approximately 2,000 feet in the headwaters to around 1,000 feet at the confluence with the Tygart Valley River in Grafton, WV. The dominant land use within the watershed is forested, which covers about 80% of the watershed. The remaining 20% of land cover is a mixture of pasture/hay, low intensity residential, open ground, etc. (2011 NLCD).

The mainstem of Three Fork Creek is approximately 19 miles in length. It begins at the confluence of Fields Creek and Birds Creek near Browns Mill, WV and flows in a general south-west direction to the confluence with the Tygart Valley River. The four largest tributaries of Three Fork Creek are Raccoon Creek (11,779 ac), Fields Creek (10,608 ac), Birds Creek (10,858 ac), and Laurel Creek (8,046 ac). The Raccoon Creek and Birds Creek sub-watersheds are the biggest contributors of AMD to Three Fork Creek. These sub-watersheds contain approximately 9,100 acres of mine pools from the Upper Freeport, Middle Kittanning and Bakerstown coal seams. These seams typically have a higher sulfur content which leads to the creation of AMD. These seams typically have higher metal content as well.

3.0 Study Area

3.1 Treatment Stations

The AML program has successfully installed four treatment stations in the Three Fork Creek watershed that release alkaline material into streams that neutralize the effects of acid mine drainage. These treatment stations apply either calcium oxide or calcium hydroxide to the affected water using stream water to actuate release mechanisms that dispense the material. Figure 19 in Appendix A shows a typical design for a treatment station. These stations are located on four tributaries of Three Fork Creek where data has shown substantial contributions of AMD have negatively affected the watershed. These streams are Birds Creek, un-named tributary (UNT) of Birds Creek at river mile 0.64, Squires Creek, and Raccoon Creek. Figure 21 of Appendix B shows the treatment station locations. Table 5 in Appendix B includes Lat/Long coordinates for the treatment stations.

3.2 Assessment Stations

The WAB established four main sample stations along Three Fork Creek to monitor the effectiveness of AMD treatment. Figure 21 in Appendix B includes a map with watershed location, sample sites, and treatment station locations. Table 4 in Appendix B includes Lat/Long coordinates for the sample stations. Stations were established at mile points 0.4, 5.7, 9.62, and 17.4. Water samples, habitat assessments, benthic macroinvertebrate collections, and electrofishing surveys were performed at each of the stations. Continuous water quality data loggers were deployed periodically throughout a five-year period ranging from 2009 to 2014 at mile points 5.7 and 17.4. A primary sample location, mile point 9.62, and secondary sample location, mile point 18.6, was used for a sediment/precipitate sample in 2012. Appendix A contains photos of the sample stations on Three Fork Creek.

4.0 Methods

4.1 Methods Information

The methods used for this study follow standard operating procedures established by the WAB. Most of the methods are modified versions of US EPA’s Rapid Bioassessment Protocol (RBP; Barbour et al. 1999). A more detailed description of WAB methods can be found at the website below.

http://www.dep.wv.gov/WWE/watershed/Pages/WBSOPs.aspx

Strict adherence to these methods was enforced for all assessment activities including the pre- and post-treatment sampling dates. Therefore, any positive changes in stream condition observed between sample dates may be more appropriately attributed to real water quality improvements and not to variation(s) in sampling methods.

4.2 General Layout

Before samples were collected or assessments conducted, an assessment reach was established at each station. The length of the reach was dependent upon the assessment activity to be performed. For example, a standardized 100-meter reach was established for benthic macroinvertebrate sample stations. Fish assessment reaches were longer and established by multiplying the average channel width at the assessment site by 40, with a maximum length of 500 meters. Field water quality readings and water samples were collected at the downstream terminus of each assessment reach. Habitat assessments, benthic macroinvertebrate collections, fish collections, and other evaluations were made throughout the entire reach.

4.3 Habitat Evaluation

A habitat evaluation was conducted utilizing a modified version of U.S. Environmental Protection Agency's Rapid Bioassessment Protocol. The approach focuses on integrating information from specific parameters on the structure of the physical habitat that are important to the survival and maintenance of benthic macroinvertebrate and fish populations and communities. Ten parameters were evaluated and given a score on a scale of 0 to 20. The scoring is broken down into four categories: 0 to 5 = Poor, 6 to 10 = Marginal, 11 to 15 = Suboptimal, 16 to 20 = Optimal. The ten scores were summed to provide a total habitat score for each station (maximum score = 200). Total score condition categories are: 0 to 59 = Poor, 60 to 109 = Marginal, 110 to 159 = Suboptimal, 160 to 200 = Optimal.

4.4 Physico-chemical Samples

For single sample discrete visits, a multi-probe (YSI Brand) was used to determine field measurements of dissolved oxygen (mg/L), water temperature (°C), pH (Std. Units), and conductivity (μmhos/cm). Water samples (mg/L) were collected at each assessment station and returned to Pace Analytical Laboratory for analysis of total metals (aluminum, calcium, iron, magnesium, manganese, potassium, selenium, sodium), dissolved metals (aluminum, copper, iron, zinc), and other constituents (hardness, hot acidity, alkalinity, chloride, sulfate, total dissolved solids, total suspended solids, nitrate-nitrite nitrogen, TKN, total nitrogen, total phosphorus). Only pH, alkalinity, hot acidity, total and dissolved aluminum, and total manganese are presented and summarized in this report. Results of other parameters are available from WAB upon request.

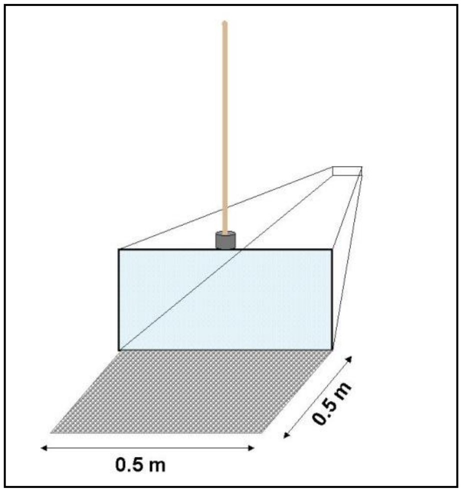
In general, deployable dataloggers were installed in early spring (March) and maintained at the monitoring site until late fall (November). Monthly visits were made to each datalogger for maintenance, calibration, data downloads, and re-installation. An instantaneous reading was also recorded during the monthly visit in order to obtain discrete checks for correcting the logged data, if required. The dataloggers were programmed to record hourly readings of dissolved oxygen (mg/L), water temperature (°C), pH (Std. Units), and specific conductance (μmhos/cm).

4.5 Metal Precipitates and Embeddedness

A concerted effort was made to evaluate the extent of metals precipitation/staining and embeddedness resulting from the instream dosers and sand dumps. The intensity of metals precipitation/deposits (Al-Aluminum, Fe-Iron, Mn-Manganese) was evaluated within the assessment reach by visually rating them as 0 = None, 1 = Low, 2 = Moderate, 3 = High, 4 = Extreme, and by estimating substrate embeddedness and sediment deposition using USEPA's Rapid Bioassessment Protocols. Photos of the substrate at these sites were also used to evaluate whether there was an increase in precipitate deposition for pre- and post-treatment visits.

4.6 Benthic Macroinvertebrate Samples

The following are standard protocols utilized by WAB. In general, they represent a slight modification of the U.S. Environmental Protection Agency's protocol for conducting biological assessments of streams and rivers.



Benthic macroinvertebrates were collected using a 0.5 meter wide rectangular frame kick net with 500 µm mesh openings. The bottom substrate was examined to ensure that habitat was similar at each collection station. The net was positioned on the stream bottom in a riffle/run area so as to eliminate gaps under the frame. The surfaces of all large substrate particles (large gravel and larger) were cleaned using a dish washing scrub brush. The substrate particles were held in front of the net while brushing all surfaces so that dislodged organisms flowed into the net. Cleaned substrate particles were then set aside and the substrate was kicked vigorously for 20 seconds in an area approximating 0.25 square meters (one net width wide by one net width upstream of the net). This action dislodged bottom dwelling organisms and washed them into the net. Four kick samples were collected at each site and composited into one sample that represented approximately 1 square meter of stream bottom substrate. The samples were preserved in 95% ethanol and returned to WAB's biology laboratory for sorting and identification. Sorting involved placing the entire benthic sample into a rectangular sorting tray and removing a 200 organism sub-sample. The organisms were identified to genus or lowest level possible.

In order to determine the health of each station both pre- and post-treatment, an Index of Biotic Integrity score (IBI) called the WVSCI was calculated for each benthic sample. The WVSCI is an IBI built on family-level identifications of benthic macroinvertebrates. The WVSCI is a good tool for detecting obvious impacts, as well as identifying the subtle effects of changing water quality conditions like those in AMD restoration studies.

4.7 Fish Community Samples

The community of fish species residing at each station was sampled using a standardized wadeable stream, electroshocking technique established by WAB. The four Three Fork Creek stations were established by following a 40 x the average channel width methodology. Thus, a 400 m stream reach was electroshocked at mile 0.4, a 300 m reach at mile 5.7, a 300 m reach at mile 9.62, and a 270 m reach at mile 17.4. A Smith-Root, Inc. tote barge and Smith-Root, Inc., backpack electrofishing unit(s) was used to collect fish by beginning at the downstream end of each reach and slowly proceeding in an upstream direction alternating bank to bank, including all side channels and backwater pools. Figure 20 of Appendix A shows a tote barge electrofishing setup. The technique involves a thorough sampling of all available habitats (riffles, runs, pools) and netting all fish observed for placement into a temporary holding bucket for identification and enumeration. All fish specimens that were positively identified in the field were processed, enumerated, and released if they were in suitable condition (*i.e.,* not dead or dying) except those that were retained for voucher or reference collections. Specimens retained for voucher or reference collections were placed in a one-gallon Nalgene container, appropriately preserved in a formalin-based solution, and returned to the laboratory to be identified and enumerated. The species lists and counts for the field released specimens were then added to the ones processed in the laboratory to obtain final results for each station.

The health of the fish community at each station was evaluated and compared by examining species composition, species diversity, fish abundance, and pollution tolerance.

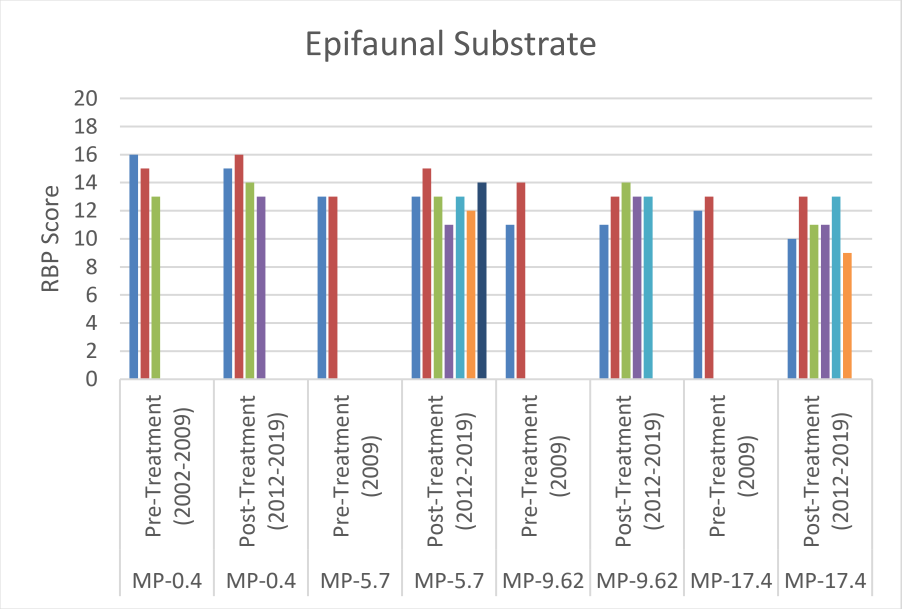


Figure 1-Pre- and post-treatment epifaunal substrate RBP ratings.

5.0 Results/Discussion

5.1 Habitat Evaluation

Since 2009, 31 individual RBP habitat surveys were performed by various members of the WAB staff between the four primary sample stations. Each comprehensive habitat survey was completed using WAB protocol. In general, habitat conditions have remained relatively stable at each of the sample stations throughout the course of the sampling period. It is important to note that streams are dynamic in nature and variabilities exist from sample to sample in terms of RBP habitat scores. Seasonal flow rates often drive sedimentation, embeddedness, and epifaunal substrate scores.

The epifaunal substrate/fish cover category is the measure of the quality of physical habitat within a stream. There were no notable differences in pre- and post-treatment samples at the 0.4, 5.7, and 9.62 sample stations. Figure 1 compares epifaunal substrate/fish cover scores between pre- and post-treatment data. Each of these sample stations exhibited natural variability between sample visits.

Typically, epifaunal substrate values were in the low sub-optimal range. Some slight differences between pre- and post-treatment epifaunal substrate scores were exhibited at station 17.4. Pre-treatment values fell within the middle sub-optimal category, but post-treatment samples tended to fall toward the low suboptimal category and a few samples ended up in the upper marginal category. In 2019, the epifaunal substrate score at mile 17.4 was 9. One potential reason for the lower epifaunal score in 2019 could be related to stream flow and the proximity to treatment upstream. The summer of 2019 was a very dry period in which stream flows were very reduced. This sample station is only a few miles downstream of the treatment systems. These zones are often areas where deposition of metal precipitates occur from upstream treatment. When flows are low, metal precipitates along with sediments are not transported downstream as readily. Sediment and precipitates settle on stream substrate and begin filling interstitial space which in turn, lowers the epifaunal substrate score.

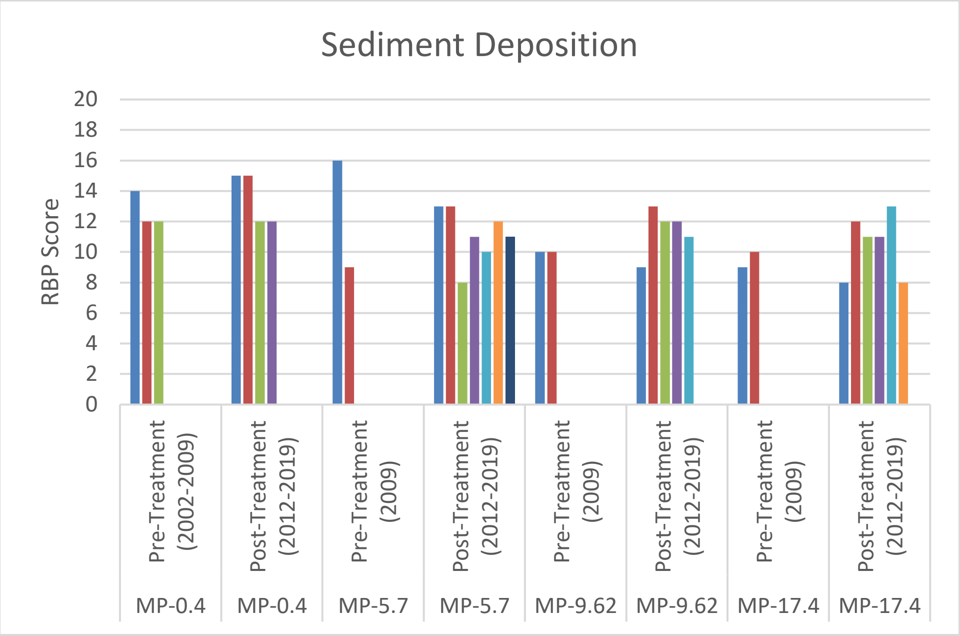


Figure 2- Pre- and post-treatment sediment deposition RBP ratings.

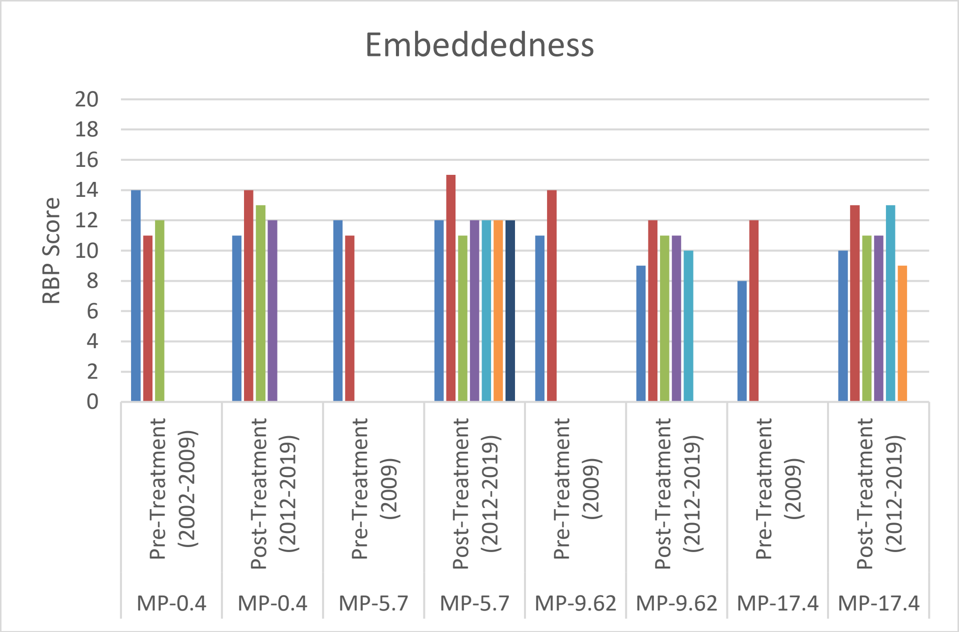


Figure 3- Pre- and post-treatment embeddedness RBP ratings.

The <u>sediment deposition category</u> measures the amount of fine sediments that accumulate within typical depositional areas. This category focuses primarily on pool and run habitat deposition. Increased sediment deposits can be detrimental to aquatic organisms and serve to decrease habitat complexity. In general, there were no notable differences between pre- and post-treatment sediment deposition scores. Figure 2 displays the results of pre- and post-treatment sediment deposition scores. Sediment deposition scores at all the sample stations tended to fall in the low to mid sub-optimal range, with some visits scoring in the mid to upper marginal category. Similar to epifaunal substrate, sediment deposition scores can be variable based on seasonal flow rates as well as human disturbances in the watershed above the sample stations.

The embeddedness category is a measure of the amount of interstitial space within riffle and run habitats. This is a very important category because it directly impacts the amount of usable space that benthic macroinvertebrates and benthic fishes have to function. In general, there were no significant differences between pre-and post-treatment embeddedness scores. Figure 3 shows a comparison of these scores. This category responds similarly to epifaunal and sediment deposition in terms of seasonal variability and flow dynamics.

5.2 Physico-chemical Samples

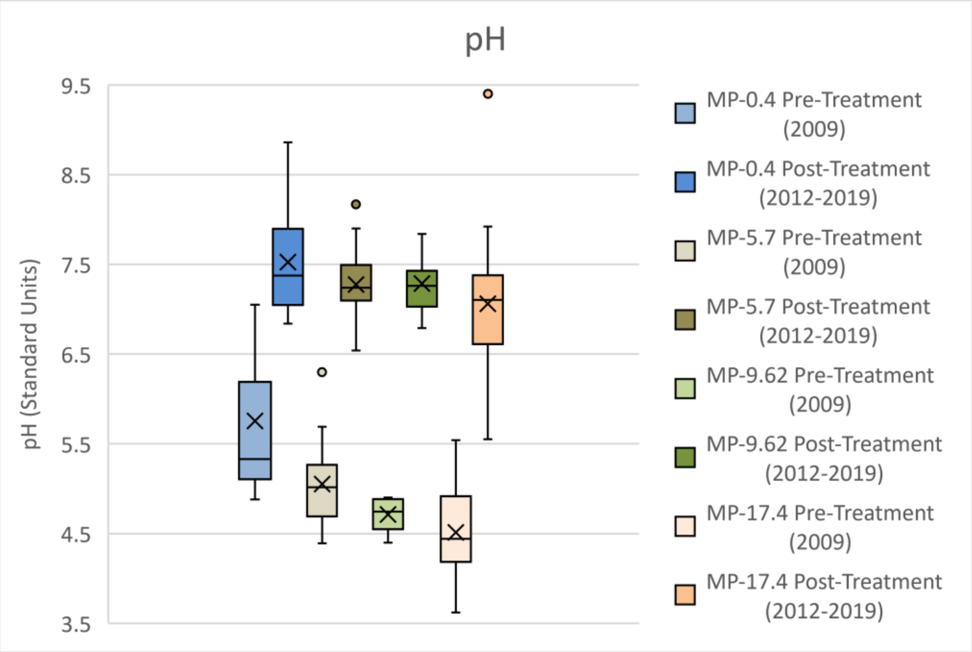


Figure 4- Pre- and post-treatment pH results.

Since 2009, WAB staff have collected 177 individual samples among the four sample stations. These samples vary from simple field parameter discrete readings to comprehensive lab water quality analyses. Available data collected by AML staff has also been included within the dataset. It is important to note that there are fewer pre-treatment samples from the stations. This does not allow for a comprehensive review of treatment effectiveness. However, some general inferences can be made about some of the major parameters that have negatively influenced water quality. Overall, water quality has been greatly improved by the installation and operation of the treatment systems.

One area of improvement has been the increase in pH values. All sample stations showed a significant increase in pH values between pre- and post-treatment sampling (Figure 4). The Median value for pre-treatment samples taken at mile point 0.4 was 5.33 pH. Pre-treatment sample medians at the other three stations were at least a pH of 5.0 or below. A very significant increase in pH sample medians occurred after treatment began. Post-treatment sample medians for all sample stations were at least a pH of 7.1 and higher.

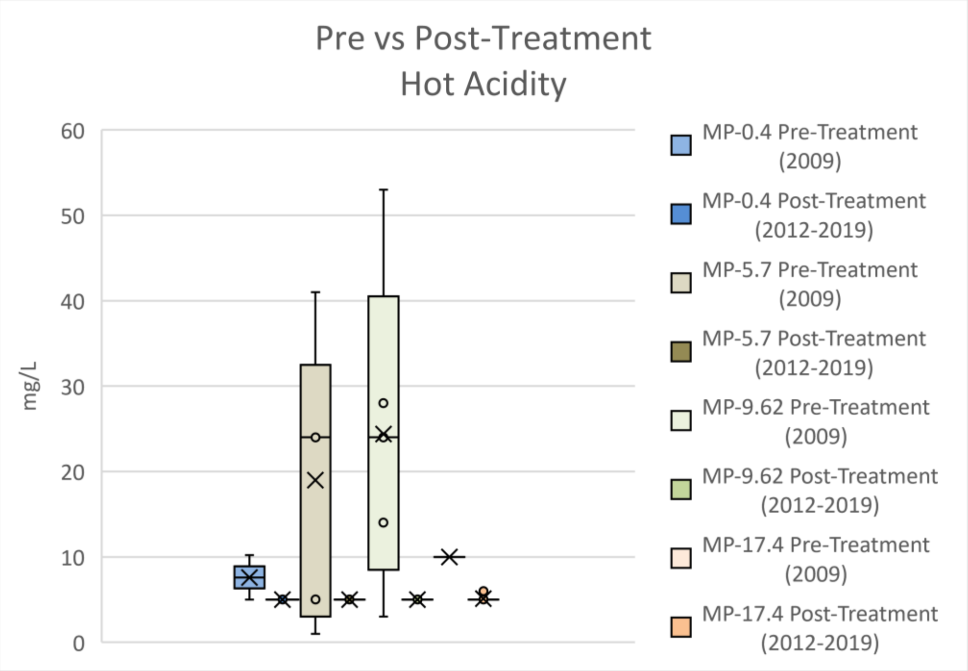


Figure 5- Pre- and post-treatment hot acidity results.

Along with the increases in pH, hot acidity values were also substantially reduced (Figure 5). This was especially apparent at stations 5.7 and 9.62. Median values for pre-treatment samples at these stations were both at 24 mg/L concentrations. Post-treatment sample medians were at the minimum detection limit (MDL) of 5 mg/L. Station 0.4 and 17.4 also saw reductions of hot acidity concentrations from medians of 7.6 mg/L and 10 mg/L, respectively, to the MDL of 5mg/L.

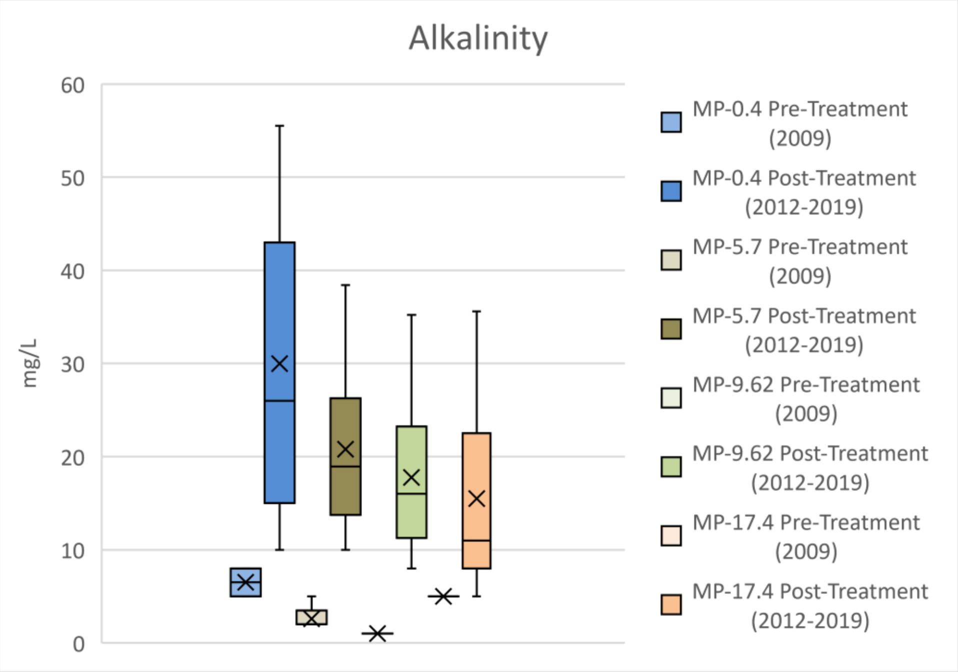


Figure 6- Pre- and post-treatment alkalinity results.

Alkalinity measures the amount of alkaline material in a water sample. This basically is a way to measure the stream’s ability to neutralize acidic conditions. Pre-treatment median alkalinity at the station 0.4 was 6.5 mg/L. All other sample stations exhibited median concentrations of 5 mg/L or less. The introduction of calcium oxide and calcium hydroxide as treatment media has significantly increased alkalinities at the sample stations. Median alkalinities at stations 0.4, 5.7, 9.62, and 17.4 were 26 mg/L, 18.95 mg/L, 16 mg/L, and 11mg/L, respectively. Figure 6 is a box and whisker plot illustrating the differences between pre- and post-treatment data.

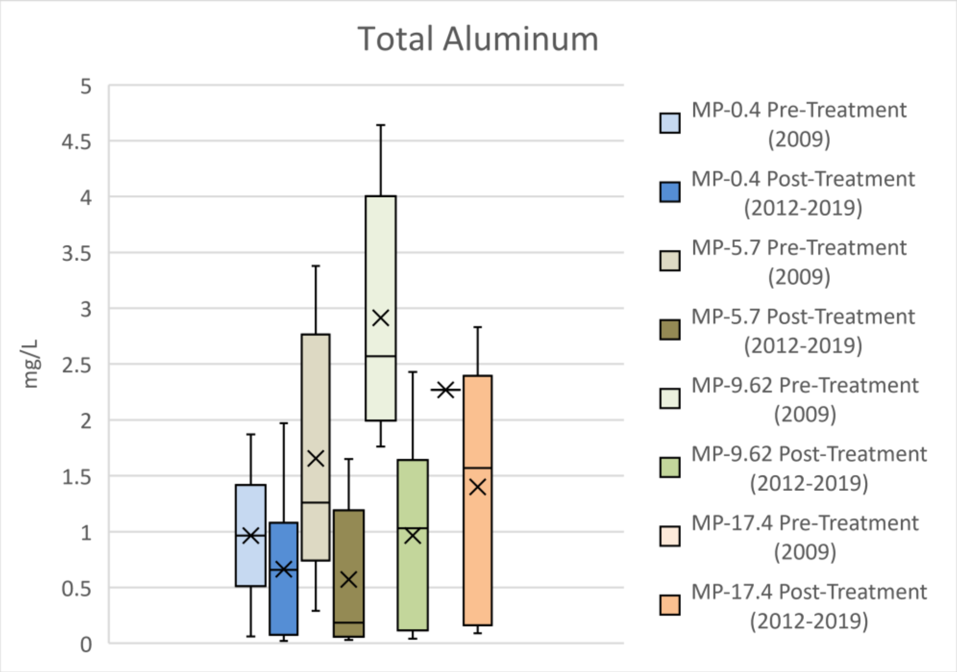


Figure 7- Pre- and post-treatment total aluminum results.

Metals toxicity in AMD impacted streams is very evident. Acidic water leaches various metals out of parent rock material which can be extremely detrimental to aquatic organisms. Pre-treatment samples from Three Fork Creek exhibited high levels of metals, most notably aluminum and manganese. Median pre-treatment total aluminum concentrations exceeded 2.5 mg/L. Some individual samples, such as one taken at mile point 9.62, were well over 4 mg/L concentration. It is important to note that the MDL for total aluminum is 0.02 mg/L. Post-treatment samples revealed varying levels of total aluminum concentrations, however reductions in total aluminum were observed (Figure7).

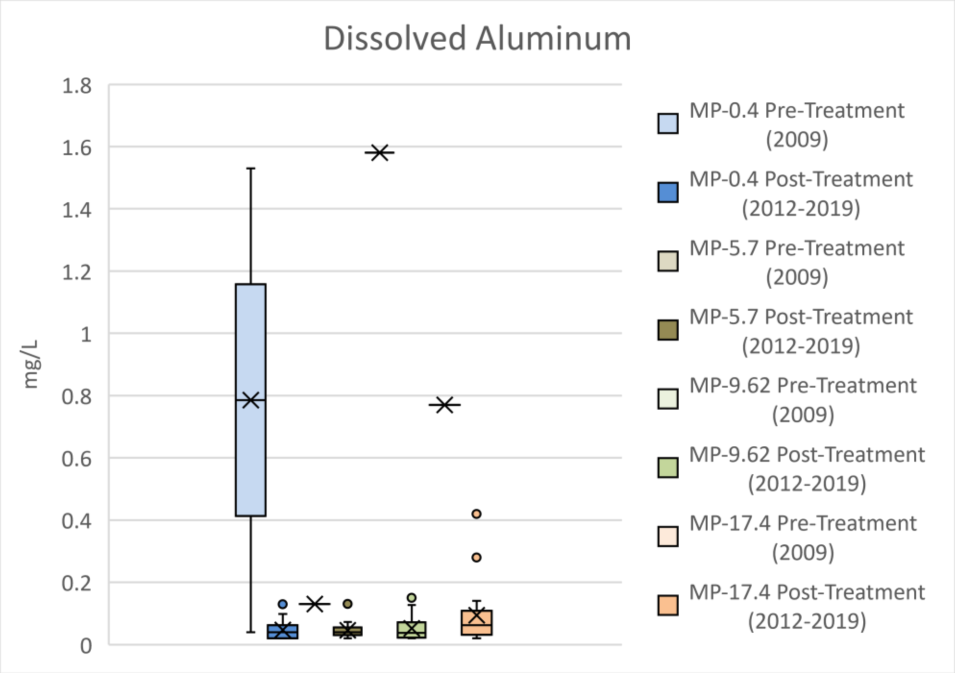


Figure 8- Pre- and post-treatment dissolved aluminum results.

One possible explanation for the post-treatment variability of total aluminum levels could be related to total suspended solids (TSS). Metals such as iron, aluminum, manganese, etc. are present in soils and sediment. These metals are often chemically bound to sediments. These compounds are typically inert and are not bio-available to aquatic organisms. At times of higher stream flows, increased TSS values are typically observed. The increased TSS values are most often from sediments being transported in the water column. To test for total metals, water samples are acidified to pH values below 2 which dissolves all sources of aluminum. This includes aluminum that is not bio-available to aquatic organisms. Dissolved forms of aluminum are highly toxic to aquatic organisms as well.

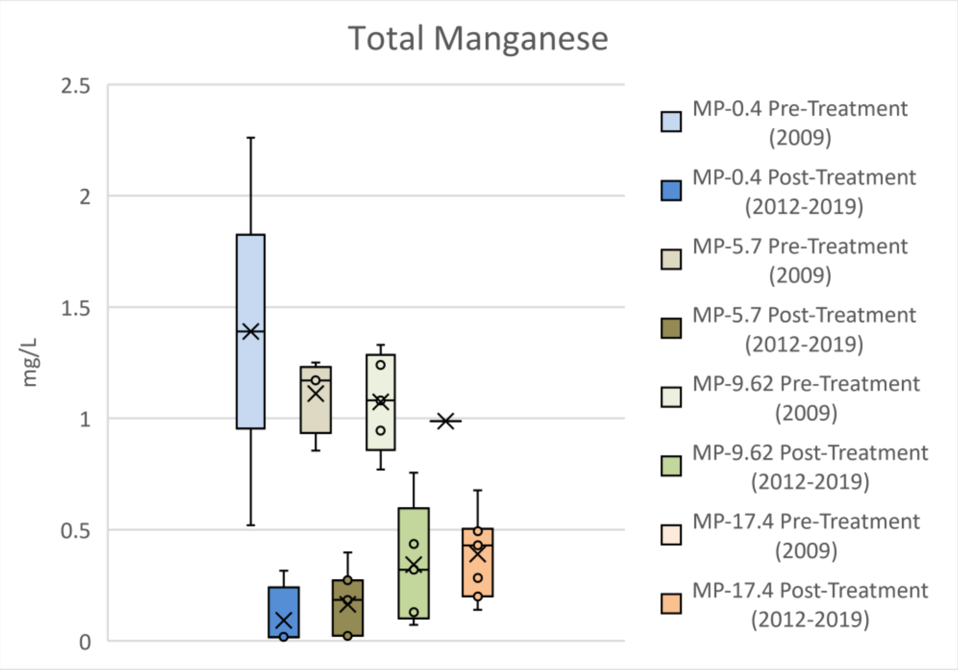


Figure 9- Pre- and post-treatment total manganese results.

Only five pre-treatment samples, two from station 0.4 and one from the remaining stations, exist in the pre-treatment dataset. This does not allow for a comprehensive review of this constituent, but the data is useful, nonetheless. Of the five pre-treatment samples, the highest levels of dissolved aluminum were observed at station 0.4 and station 9.62. Station 0.4 had a concentration of 1.53 mg/L and the station 9.62 exhibited a concentration of 1.58 mg/L. Post-treatment median concentrations of dissolved aluminum for stations 0.4, 5.7, 9.62, and 17.4 were 0.04 mg/L, 0.04 mg/L, 0.038 mg/L, and 0.062 mg/L, respectively. Figure 8 is a comparison of pre- and post-treatment dissolved aluminum concentrations.

High manganese concentrations can also have a detrimental impact on aquatic organisms. Pre-treatment concentrations of manganese tended to be high. The highest concentration from the dataset was collected at mile 0.4 at a concentration of 2.26 mg/L. All other stations exhibited high pre-treatment concentrations as well. Post-treatment sample concentrations were found to be significantly lower (Figure 9).

Continuous water quality data using deployable meters was collected at two sample locations from 2009 to 2014. Data loggers were placed at stations 5.7 and 17.4 and recorded pH values every hour, typically from April to November. Continuous monitoring data is more powerful than single-day grab values for most parameters, especially pH, a parameter that can exhibit substantial daily and seasonal variability. Data from the deployable logger at mile 5.7 showed high variability in pre-treatment pH values (Appendix C, Figure 22). Swings of 4.5 to 7.5 occurred often. When treatment began in April of 2011, data shows some initial high variability, but pH ranges tightened up after a few months of operation and rarely dropped below a value of 6.5 after that. The period of variability after treatment began was most likely related to treatment station calibration.

The data logger at the station 17.4 showed similar characteristics (Appendix C, Figure 23). Pre-treatment data showed fluctuations between a pH value of approximately 4 to values in the 6.5 range. After treatment began in April of 2011, a similar period of variability followed, then transitioned into a tighter range between a pH of 7 and 8. It is interesting to note that in 2013 and 2014 highly variable pH characteristics were observed. There were a few large spikes and declines of pH values during these years. One reading spiked to a pH of over 10 and a few declines to a pH in the 5 range. One decline even extended into the high 4 range. One possible explanation for the times of high variability could be related to stream flow and proximity to upstream treatment stations. Examination of USGS stream gage data showed highly variable flows during these deployment periods. It is surmised that because the treatment stations operate based on stream flow, that these high flow periods could have caused the variability of pH values. Some issues with stream debris obstructing the intakes of treatment stations have been seen, causing the facility to function improperly. Also, the proximity of this sample station to three treatment stations likely exacerbated the effects.

5.3 Metal Precipitates

Streams affected by acid mine drainage often have elevated levels of metal precipitates (i.e. iron, aluminum, manganese). When the water is neutralized during treatment, the extent of metals precipitating to the stream bottom may intensify. Negative effects to aquatic life include reduced visibility, blanketing the bottom so that it smothers benthic organisms, and filling in the places they live and search for food. Therefore, WAB made a concerted effort to monitor potential changes in the levels of precipitates following the start of treatment. Many physical and chemical processes are involved in precipitation of metals in streams, so varying levels from one site visit to the next is expected.

Sample Date	Mile Point	Fe Hydroxide Intensity	Al Hydroxide Intensity	Mn Hydroxide Intensity
8/17/2009	**0.4**	2	0	0
9/29/2009	**0.4**	2	3	1
8/29/2012	**0.4**	1	0	0
8/20/2013	**0.4**	1	1	2
8/29/2016	**0.4**	1	0	2
9/3/2019	**0.4**	1	0	1
8/17/2009	**5.7**	2	2	0
9/29/2009	**5.7**	1	2	0
8/29/2012	**5.7**	1	0	0
8/20/2013	**5.7**	1	0	1
8/30/2016	**5.7**	1	0	2
9/4/2019	**5.7**	1	0	2
8/18/2009	**9.62**	1	2	0
9/30/2009	**9.62**	2	2	0
8/30/2012	**9.62**	1	0	0
8/20/2013	**9.62**	2	0	2
8/30/2016	**9.62**	2	1	1
9/4/2019	**9.62**	2	0	2
8/18/2009	**17.4**	2	2	0
9/30/2009	**17.4**	2	2	0
8/30/2012	**17.4**	1	0	0
8/20/2013	**17.4**	2	0	2
8/31/2016	**17.4**	2	0	1
9/5/2019	**17.4**	2	0	1

Table 1-Metal precipitate intensity ratings. Pre-treatment samples are highlighted in white and post-treatment samples are highlighted gray.

In general, metal hydroxide levels declined somewhat at most stations. Aluminum hydroxide at all stations dropped significantly between pre- and post-treatment surveys (Table 1). Most stations exhibited an aluminum hydroxide intensity of 2 for pre-treatment surveys. Post-treatment survey data shows a decline in this hydroxide intensity to a non-observable level. Stations 0.4 and 5.7 showed a decrease in iron hydroxide intensities from 2 to 1. Stations 9.62 and 17.4 showed no notable changes in iron hydroxide intensities. This is likely due to the proximity of AMD impacted streams and treatment stations in relation to the sample stations. Manganese hydroxide levels exhibited increases in intensities between pre- and post-treatment surveys.

Three Fork Creek Precipitate Analysis
Mile 9.62

TD	*Analyte*	*Qualifier*	*Result*	*MDL*	*Units*
Total	Iron		106	0.02	mg/L
Total	Aluminum		88.7	0.02	mg/L
Total	Manganese		10.9	0.003	mg/L
Total	Magnesium		5.8	0.2	mg/L
Total	Calcium		7.8	0.2	mg/L
Total	Zinc		4.29	0.005	mg/L
Total	Potassium		1	0.5	mg/L
Total	Sodium	<	0.5	0.5	mg/L
Total	Copper		0.092	0.003	mg/L
Total	Beryllium		0.0616	0.00005	mg/L
Total	Selenium		0.0013	0.001	mg/L

Table 2-Precipitate analysis for the mile 9.62 station.

After treatment began, stations 9.62 and 17.4 began to develop areas with an increased amount of precipitate deposition. Especially in years with low flows, this precipitate was noted in slow moving areas and tended to create a thick blanket over the substrate in these areas. WAB staff collected this material at one primary sample location, mile 9.62, and one secondary station, mile 18.6, and had it analyzed by a laboratory (Tables 2 and 3). Analysis found that it was a mixture of inorganic sediments, periphyton, algae, and metals. This is a byproduct of the treatment process that can have negative impacts on benthic macroinvertebrate communities and benthic fishes.

Three Fork Creek Precipitate Analysis
Mile 18.6

TD	*Analyte*	*Qualifier*	*Result*	*MDL*	*Units*
Total	Iron		92	0.02	mg/L
Total	Aluminum		75.6	0.02	mg/L
Total	Manganese		12.1	0.003	mg/L
Total	Magnesium		12.4	0.2	mg/L
Total	Calcium		10.5	0.2	mg/L
Total	Zinc		2.09	0.005	mg/L
Total	Potassium		0.7	0.5	mg/L
Total	Sodium	<	0.5	0.5	mg/L
Total	Copper		0.083	0.003	mg/L
Total	Beryllium		0.0354	0.00005	mg/L
Total	Selenium		0.0013	0.001	mg/L

Table 3-Precipitate analysis for the mile 17.4 station.

5.4 Benthic Macroinvertebrate Sampling

A total of 27 individual benthic macroinvertebrate samples were collected among the four sample stations since 2009. Only five pre-treatment samples were collected among the four sample stations. All stations had at least one sample taken except at mile 0.4, which had a previous sample from 2002 that was incorporated into this report to help boost the dataset.

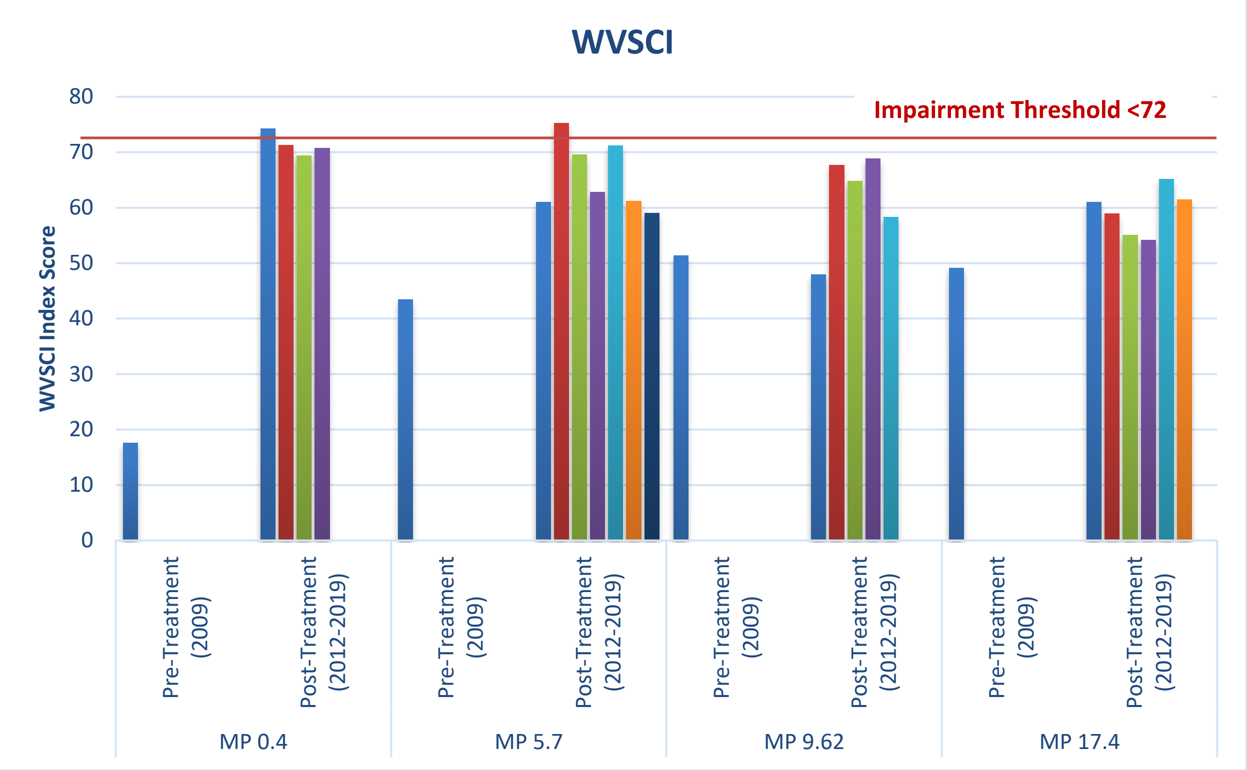


Figure 10-Pre-and post-treatment WVSCI scores.

WAB uses the West Virginia Stream Condition Index (WVSCI) to assess benthic macroinvertebrate communities. This is a multi-metric index that uses macroinvertebrates to determine the health of a stream. Because larval macroinvertebrates are relatively stationary, they are very susceptible to changes in water quality. This makes them an excellent indicator for stream health. It is important to note that benthic communities are very complex and are susceptible to many environmental factors including stream discharge, stream habitat in relation to sedimentation, localized disturbances, and even life history strategies of different genera. In general, WVSCI scores improved at all stations following the treatment of AMD in Three Fork Creek. Figure 10 shows differences in pre- and post-treatment WVSCI scores. Station 0.4 revealed the largest change in WVSCI scores within the dataset. In 2009, the WVSCI score was 17.61. When this station was sampled again in 2012, after treatment had begun, the WVSCI score was 74.25. The impairment threshold for WVSCI is 72. This means that any score less than 72 is considered impaired. Lower scores, like 17.61 for example, are considered severely impaired. Sites scoring over 72 are considered unimpaired. Subsequent years samples did not pass WVSCI, but the scores remained very close to the threshold. A similar positive trend emerged at the other sample stations. Pre-treatment WVSCI score was 43.46 and the post-treatment median was 62.86 at station 5.7. Station 9.62 had a pre-treatment score of 51.4 and the post-treatment median WVSCI score was 64.77. Station 17.4 showed the smallest increase in WVSCI score. The pre-treatment sample WVSCI score was 49.13 and post-treatment median score was 59.98.

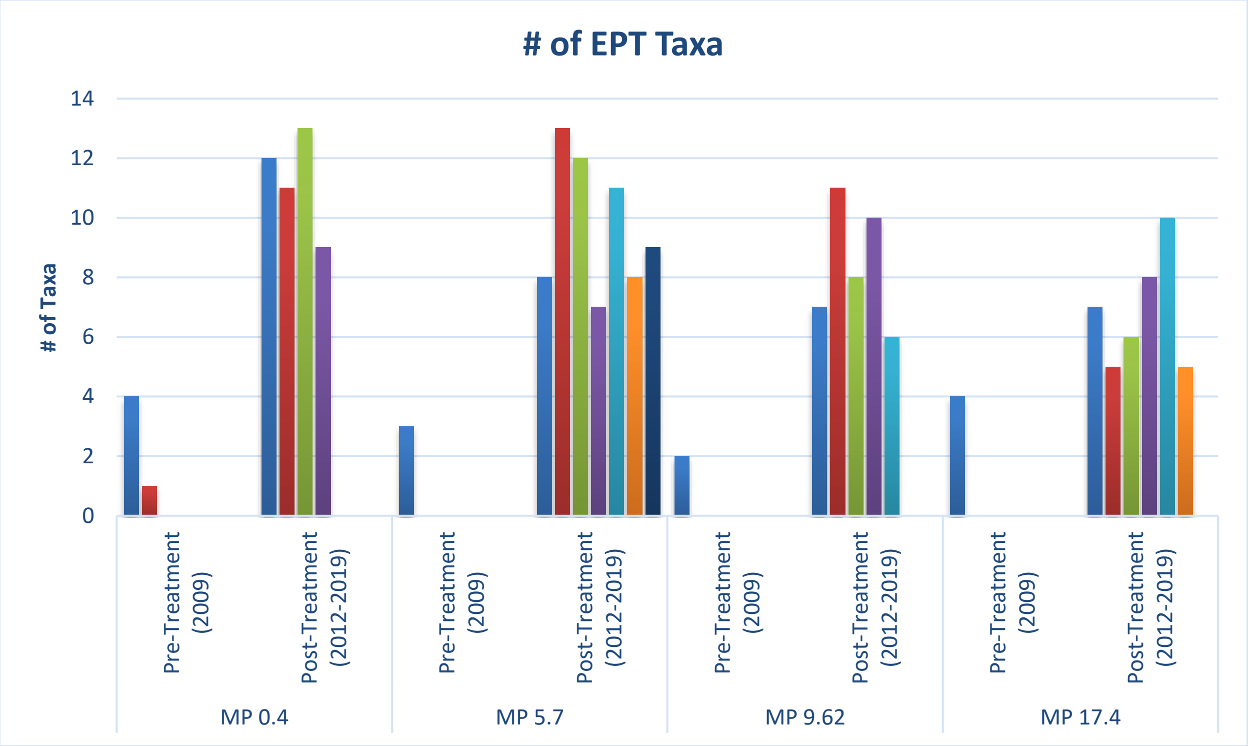


Figure 11-Pre- and post-treatment EPT taxa richness.

Another benthic metric often examined when comparing stream samples, especially pre- and post-treatment samples, are numbers of Ephemeroptera, Plecoptera, and Tricoptera (EPT) taxa. Although differences in pollution tolerances exist among these groups, EPTs are often regarded as the most sensitive to pollution. This means that degraded streams usually have a lower amount of EPTs than higher quality streams. In 2009, station 0.4 had only one EPT taxa collected in the benthic sample. Post-treatment median EPT taxa was 12. Similar post-treatment patterns arose among the other stations as well (Figure 11).

5.5 Fish Community Sampling

Since 2009, 19 individual electrofishing surveys were completed on Three Fork Creek. Of the 19 samples, four are pre-treatment samples. It is important to note that station 0.4 has only had three post-treatment samples, whereas the remaining sample stations have had four. In 2014, stream flow at the station 0.4 was very high and the fish community was unable to be assessed. During higher than normal flows, visibility is reduced which allow fish to escape collection. This may bias the numbers/species of fish collected for the sample. There are also numerous safety concerns due to the increased stream flow, consequently the station was not sampled.

Pre-treatment electrofishing surveys performed in 2009 only yielded one fish collected between the four sample stations. One green sunfish was collected at station 5.7. Stations 0.4, 9.62, and 17.4 produced no fish. Post-treatment samples from 2012 produced significant results when compared to pre-treatment samples. The survey at station 0.4 produced 950 individual fish comprised of 17 unique species. Similar results were found at the other sample stations. Station 5.7 yielded 199 individual fish belonging to 10 different species. Subsequent years of sampling at this station showed a steady rise in number of individual fish and number of species collected. In 2019, this station produced 717 individual fish belonging to 16 unique species. Station 9.62 also showed similar results in terms of numbers of individual fish, however the number of species has remained at 11. It is important to note that as one moves further upstream from a large source population (e.g. Tygart Valley River), and watershed areas become smaller, species diversity tends to decline in most situations. It is very common to have higher species richness near the confluence of a stream and a river because swim distances from source populations to the lower reaches are reduced. Seeing less species richness further up a watershed is a normal occurrence. It is important to note that in 2019 at station 9.62, two mottled sculpins were collected. Mottled sculpins are a fish species that typically inhabit cool and cold-water streams. This species is typically regarded as being moderately sensitive to certain types of pollution and sedimentation. It is very positive to see this species returning to the upper reaches of Three Fork Creek. Station 17.4 showed notable improvements to the fish community as well. In 2012, 436 fish were collected comprised of 3 different species. Subsequent years showed variability in the total number of individuals collected but species richness increased slightly. Variability in fish community structure is a normal occurrence and does not always indicate stress to the community. The variability at this station is likely just from natural population dynamics. This station also has seen a rise in mottled sculpin presence and abundance. One mottled sculpin was first collected here in 2016. In 2019, 34 mottled sculpins were collected.

WAB often displays a fish per meter (fish/m) metric when describing fish communities. This is a coarse measure of abundance and can be informative in determining the effectiveness of treatment in acid mine drainage streams where fish numbers are often diminished. Pre-treatment samples at mile 0.4, 9.62, and 17.4 had no fish which gave a fish/m metric score a 0. Having one fish at the station at mile 5.7

yielded a fish/m score of 0.003. Post-treatment samples increased the fish/m metric substantially. In 2012, the mile 0.4 station produced a 2.38 fish/m score. Subsequent years showed reductions and variable results in the fish/m scores, but this may be due to natural variability in fish community structure. Station 5.7 showed steady increases in the fish/m metric and in 2019, had the highest score of any of the stations throughout the sampling period. For comparative purposes, the median fish/m metric score for WAB Level I and II fish reference sites in the Central Appalachian ecoregion is 2.17 fish/m. Table 6 in Appendix B shows a comparison of pre- and post-treatment fish community results.

6.0 Conclusions

Based on the findings of this study, the overall health of Three Fork Creek has improved substantially as a result of AMD treatment efforts by DEP's AML program. Notable increases in physicochemical properties like pH and decreases of total and dissolved metals were realized following the onset of AMD treatment. Continuous monitoring data showed increases in hourly pH readings following treatment efforts. Additionally, and of notable importance, the observed increase in pH at these locations was sustained year to year after treatment began.

Stream habitat conditions remained fairly stable throughout the course of the study and typically scored in the mid to lower sub-optimal category for RBP habitat. Although some variability was seen within the sample stations, habitat conditions are generally favorable for aquatic organisms and do not appear to be limiting biological recovery to a large degree.

WVSCI was used to analyze and interpret benthic macroinvertebrate data during this study. Substantial improvements in WVSCI scores throughout the sample period were realized at all four sample stations on Three Fork Creek. Although improvements in WVSCI scores were attained, most samples remained below the impairment threshold of 72. Therefore, while the biological condition has improved, full recovery has not been realized at these sample stations.

Aside from water quality, the most dramatic increase witnessed from this study was the fish community response to AMD treatment. Pre-treatment fish community samples revealed that fish were unable to utilize the mainstem of Three Fork Creek. The stream was essentially dead, and no fish community even existed. Following treatment of AMD, a fish community was able to be re-established. In one year of treatment, Three Fork Creek went from having one fish collected among the four sample stations, to having 2,221 fish collected among the sample stations. Not only did the fish community respond well to initial treatment, it expanded further upstream and out of unimpacted tributaries in the subsequent years. After 8 years of treatment, the fish community has become well established throughout the watershed and appears to have become comparatively stable.

Appendix A

Figure 12-Example of iron staining at mile 9.62.

Figure 13-Example of precipitate mixture at mile 9.62.

Figure 14-Precipitate mixture collected from mile 17.4.

Figure 15-Looking upstream at mile 0.4.

Figure 16-Looking downstream at mile 5.7.

Figure 17-Looking upstream at mile 9.62.

Figure 18-Looking upstream at mile 17.4.

Figure 19-Typical design of an AMD treatment station.

Figure 20-Example of electrofishing survey using a Smith-Root Inc. tote barge.

Appendix B

Three Fork Creek Sample Station Locations		
Station	**Latitude**	**Longitude**
Mile 0.4	*39 ° 20' 20.44"*	*80 ° 0' 55.29"*
Mile 5.7	*39 ° 20' 41.0"*	*79° 56' 36.3"*
Mile 9.62	*39° 23' 25.6"*	*79° 54' 37.2"*
Mile 17.4	*39° 26' 32.1"*	*79° 50' 24.8"*
Mile 18.6 (secondary)	*39° 26' 50.8"*	*79° 49' 24.2"*

Table 4-Sample station locations.

Three Fork Creek Doser Locations		
Stream Name	**Latitude**	**Longitude**
Birds Creek	*39 ° 25' 59.23"*	*79 ° 48' 18.74"*
UNT/ Birds Creek RM 0.64	*39 ° 27' 12.84"*	*79° 48' 10.83"*
Squires Creek	*39° 28' 3.17"*	*79° 47' 34.71"*
Raccoon Creek	*39° 23' 24.06"*	*79° 47' 34.73"*

Table 5-Treatment station locations.

Station 0.4					
Species	**2009**	**2012**	**2014**	**2016**	**2019**
Green Sunfish	**No Fish Present**	**35**	**No Sample Collected**	**8**	**35**
Northern Hogsucker		**4**		**3**	**14**
Smallmouth Bass		**262**		**125**	**69**
River Chub		**67**		**24**	**16**
Rock Bass		**59**		**56**	**28**
Greenside Darter		**176**		**198**	**377**
Fantail Darter		**17**		**10**	**67**
Johnny Darter		**13**		**1**	**9**
Rosyface Shiner		**255**		**20**	**25**
Spotfin Shiner		**19**		**2**	**33**
Sand Shiner		**2**		**73**	**5**
Yellow Bullhead		**3**		**1**	**6**
Logperch		**1**		**5**	
Central Stoneroller		**8**		**6**	**10**
Bluntnose Minnow		**3**		**1**	
Blackside Darter		**1**			
Silver Shiner		**25**		**21**	**6**
Mimic Shiner					**118**
Longnose Dace					**1**
Mottled Sculpin					**1**
Total Species Collected	**0**	**17**		**16**	**17**
Total Fish Collected	**0**	**950**		**554**	**700**
Fish/m	**0.00**	**2.38**		**1.39**	**1.75**

Table 6-Fish community results for Station 0.4.

Station 5.7					
Species	**2009**	**2012**	**2014**	**2016**	**2019**
Green Sunfish	**1**	**54**	**14**	**6**	**92**
Northern Hogsucker		**1**	**22**	**17**	**9**
Smallmouth Bass		**27**	**48**	**56**	**37**
River Chub		**18**	**29**	**24**	**26**
Rock Bass		**27**	**12**	**33**	**31**
Greenside Darter		**30**	**72**	**202**	**270**
Fantail Darter			**3**	**29**	**60**
Johnny Darter				**2**	**13**
Rosyface Shiner		**28**	**12**	**109**	**45**
Spotfin Shiner				**17**	**15**
Sand Shiner				**13**	**11**
Yellow Bullhead		**1**		**2**	**4**
Logperch		**3**	**4**		
Central Stoneroller			**22**	**35**	**39**
Bluntnose Minnow		**10**	**8**	**23**	**22**
Silver Shiner			**19**	**15**	**14**
Creek Chub			**21**	**44**	**29**
Western Blacknose Dace			**1**		
Bluegill			**2**		
Spotted Bass			**1**		
Silverjaw Minnow				**1**	
Total Species Collected	**1**	**10**	**16**	**17**	**16**
Total Fish Collected	**1**	**199**	**290**	**628**	**717**
Fish/m	**0.003**	**0.66**	**0.97**	**2.09**	**2.39**

Table 7-Fish community results for Station 5.7.

Station 9.62					
Species	**2009**	**2012**	**2014**	**2016**	**2019**
Green Sunfish	**No Fish Present**	**39**	**29**	**17**	**37**
Northern Hogsucker			**4**	**31**	
Smallmouth Bass		**3**	**20**	**110**	**49**
River Chub		**2**	**10**	**3**	**23**
Rock Bass		**3**	**32**	**6**	**37**
Greenside Darter		**1**	**21**	**95**	**62**
Fantail Darter				**1**	**4**
Johnny Darter					**1**
Rosyface Shiner		**20**	**16**	**7**	
Spotfin Shiner			**1**		**1**
Sand Shiner				**2**	
Logperch			**13**		
Bluntnose Minnow		**1**			
Silver Shiner			**32**	**42**	**3**
White Sucker		**1**			
Creek Chub		**10**	**14**	**93**	**24**
Striped Shiner		**1**			
Mottled Sculpin					**2**
Saugeye Hybrid (Sander canadensis x S. vitreus)		**1**			
Total Species Collected	**0**	**11**	**11**	**11**	**11**
Total Fish Collected	**0**	**82**	**192**	**407**	**243**
Fish/m	**0.00**	**0.27**	**0.64**	**1.36**	**0.81**

Table 8-Fish Community Results for Station 9.62.

Station 17.4					
Species	**2009**	**2012**	**2014**	**2016**	**2019**
White Sucker	**No Fish Present**	**13**	**1**	**1**	**1**
Creek Chub		**377**	**259**	**377**	**266**
Western Blacknose Dace		**46**	**2**	**22**	**40**
Spotted Bass			**1**		
Largemouth Bass				**5**	
Mottled Sculpin				**1**	**34**
Total Species Collected	**0**	**3**	**4**	**5**	**4**
Total Fish Collected	**0**	**436**	**263**	**406**	**341**
Fish/m	**0.00**	**1.61**	**0.97**	**1.50**	**1.26**

Table 9-Fish community results for Station 17.4.

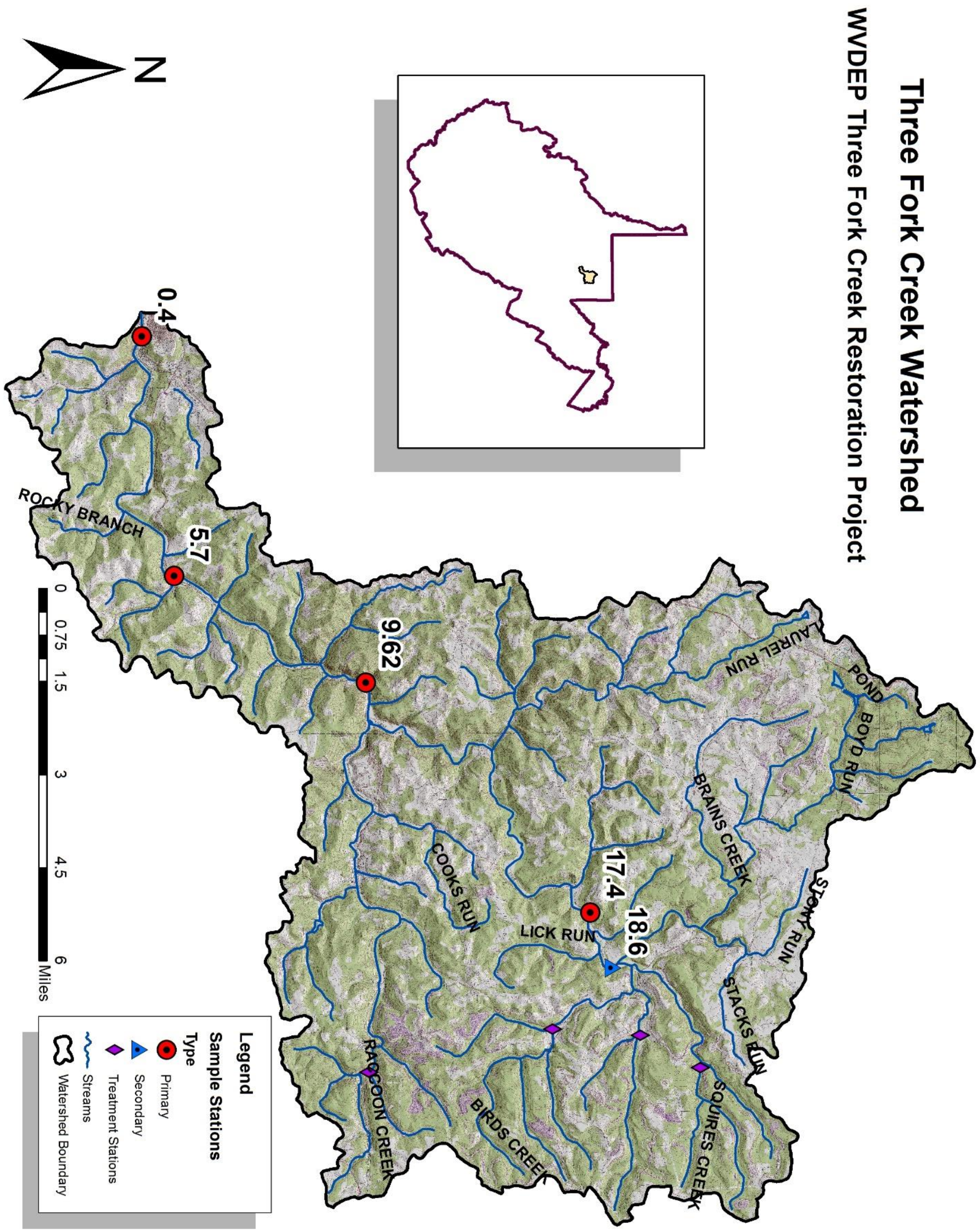


Figure 21-Three Fork Watershed study area.

Appendix C

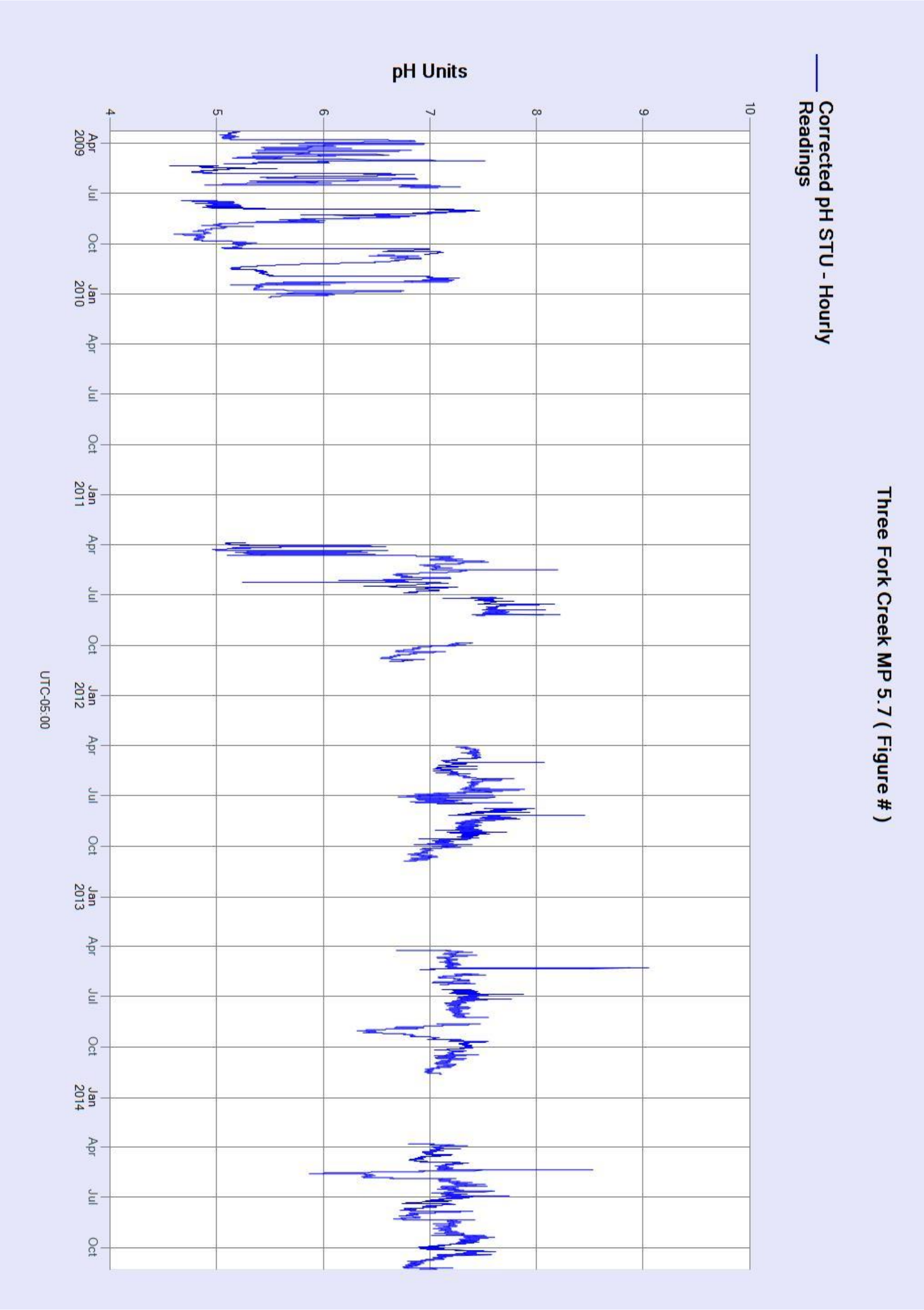


Figure 22-Continouous pH monitoring data for station 5.7.

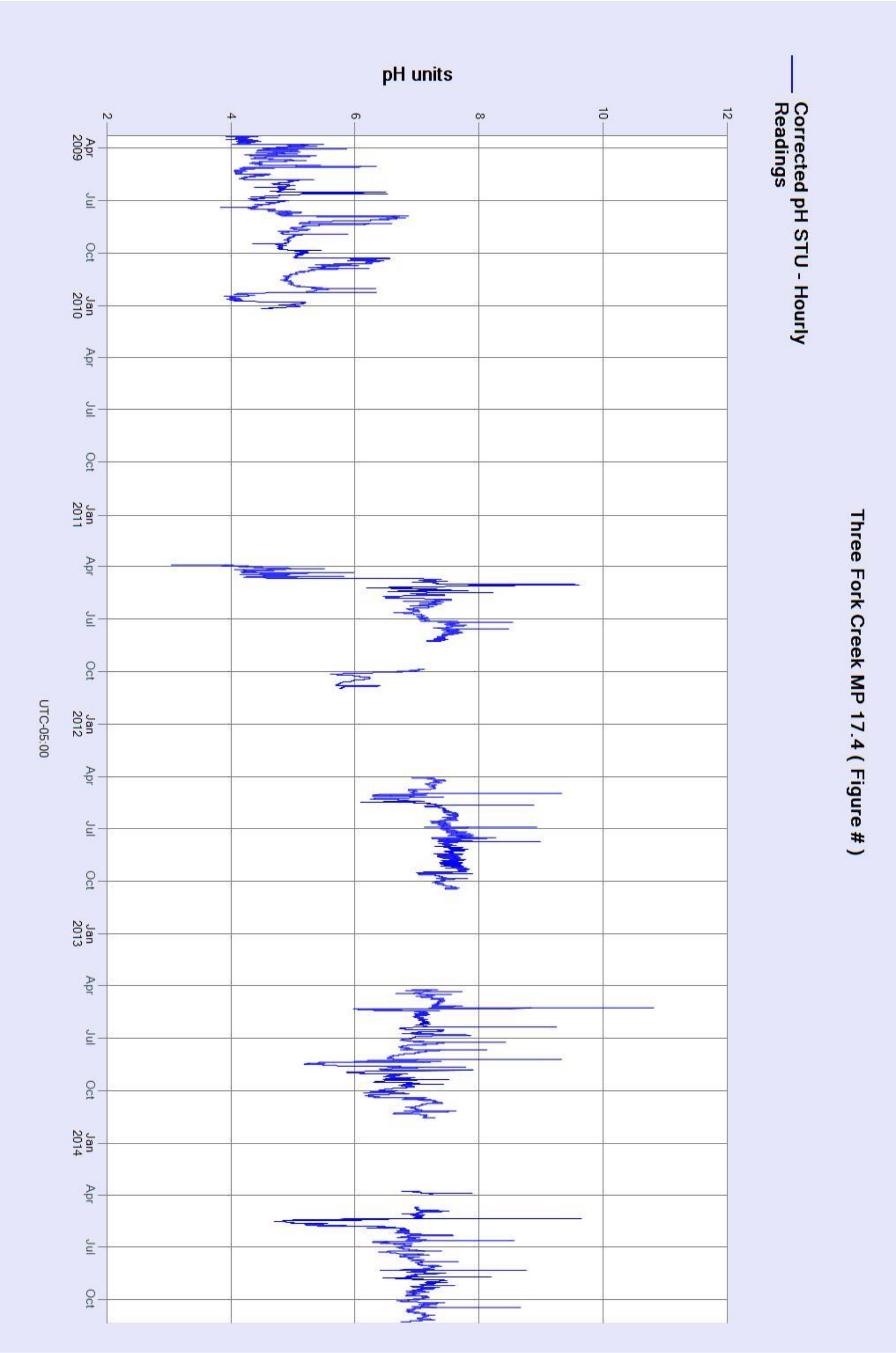


Figure 23-Continuous pH monitoring data for station 17.4.