Muddy Creek Watershed Restoration

Biological Monitoring Status Report

Prepared for:

WV Department of Environmental Protection
Division of Land Restoration
Office of Special Reclamation

Prepared by:

WV Department of Environmental Protection
Division of Water and Waste Management
Watershed Assessment Section

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Table of Contents

Table of Figures

List of Tables

1.0 Introduction

In September of 2015, the Watershed Assessment Branch (WAB) of the West Virginia Dept. of Environmental protection (WVDEP) began biological and water quality monitoring in the Muddy Creek watershed. For decades, acid mine drainage (AMD) from pre-law and post-law mining practices has negatively affected water quality in Muddy Creek. This study is part of a collaborative effort between the Office of Special Reclamation (OSR) and WAB to monitor and improve water quality within the Muddy Creek drainage. The primary objective of this project is to improve water quality on several miles of Muddy Creek. Expected benefits of improving the water quality in Muddy Creek include restoration of the benthic macroinvertebrate community and establishment of a diverse fishery within its lower reaches.

To improve water quality, a sophisticated AMD treatment facility was built along Muddy Creek to treat multiple sources of AMD. The treatment facility began operating in March of 2018. This report focuses on pre- and post-treatment data collected on Muddy Creek**. Note: this report does not include information on the recent high flow event and subsequent treatment failure at the former T & T Mine in Preston County that occurred the first week of March 2021. It will be included in future updates after all samples have been collected and analyzed.**

Watershed Description

The Muddy Creek watershed spans 21,487 acres in West Virginia. The headwaters of Muddy Creek begin in the eastern portion of Preston County, near the community of Afton. The creek flows in a general northwest direction before turning to a general southwest direction midway through the watershed, and finally to the confluence with the Cheat River near Albright, West Virginia. Land use types vary throughout the watershed. The largest land cover type being forested, encompassing over 71% of the watershed area. Grassland/pasture covers approximately 20% of the watershed area. All other land use types, including various stages of development, wetlands, and open water combine to cover 7.5 % of the watershed area.

Muddy Creek has 6 major tributaries. Martin Creek, the largest tributary and main source of AMD, encompasses an area of approximately 4,600 acres. The next five tributaries in order of watershed area, include Crab Orchard Run (1,920 ac), UNT/Muddy Creek RM 9.8 (1,790 ac), Jump Rock Run (1,148 ac), Sugarcamp Run (1,044 ac), and Sypolt Run (844 ac).

2.0 Study Area

3.1 Treatment Station Location

The WVDEP Office of Special Reclamation (OSR) facilitated the construction of a large treatment facility (T&T Treatment Station) approximately 0.5 miles downstream of the confluence of Martin Creek. The T&T treatment station utilizes alkaline materials and advanced computer systems that monitor and treat multiple sources of AMD in the nearby watershed. A lime slurry treatment facility was installed on Glade Run, a tributary of Martin Creek. This treatment station is similar to the T&T facility but lacks the large clarifying units that the T&T facility uses. Figure 13 shows the locations of the treatment facilities.

3.2 Stream Assessment Stations

The WAB originally set up three survey stations along the lower reaches of Muddy Creek to monitor baseline biotic and abiotic conditions as well as to monitor AMD treatment effectiveness (Appendix C). These stations were located at mile points 0.0, 3.3, and 4.4. In 2019, WAB staff relocated the sample station at mile 3.3 downstream to mile 2.1. This station was moved downstream to remain below the treatment facility. Benthic data collected by OSR personnel and Friends of Cheat watershed group at Station 3.3 in 2019 will be presented in this report. WAB samples from 2015 at this station will also be reported. It is important to note that the samples at Station 3.3 are above the treatment facility and will not show positive WQ, fish and benthic macroinvertebrate trends. Benthic macroinvertebrate and fish surveys, along with water quality samples and comprehensive habitat surveys were completed at Stations 0.0, 2.1, and 4.4. Station 4.4 is above the main AMD disturbances within the watershed and was used as a control station for comparison purposes. A continuous datalogger was also deployed at MP 0.2. This logger monitors temperature, pH, and specific conductance on an hourly basis.

Data from previous sampling events in the watershed are incorporated in this report to boost the dataset. Specifically, samples taken in 2006-2007 from Stations 0.0, 2.1, and 4.6 are used in the pre-treatment dataset. Station 4.6 data were incorporated with data from Station 4.4 due to proximity of the two stations. All water quality, habitat, and benthic macroinvertebrate data from Station 4.6 will be reported as Station 4.4 for ease of reporting.

3.0 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 will be 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 (RBP). 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. Only three of the ten habitat parameters are discussed in this report.

4.4 Physico-chemical Samples

For single sample discrete visits, a multi-probe meter (YSI Brand) was used to determine field measurements of dissolved oxygen (mg/L), water temperature (°C), pH (Std. Units), and specific 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, hot acidity, alkalinity, total aluminum, total manganese and total iron are presented and summarized in this report. Results of other parameters are available from WAB upon request.

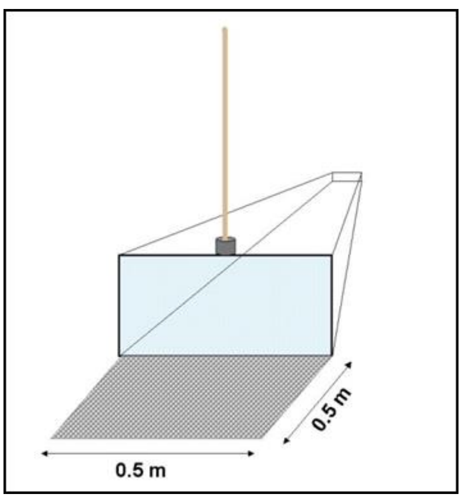
A deployable datalogger was placed at mile 0.2 to collect continuous pH readings within Muddy Creek from March 2018 to January 2019. Monthly visits were made to the datalogger for maintenance, calibration, data downloads, and re-installation. An instantaneous reading was also recorded during the monthly visit in order to obtain discrete checks for correcting the logged data, if required. The dataloggers were programmed to record hourly readings of pH (standard units).

4.5 Metal Precipitates and Embeddedness

A concerted effort was made to evaluate the extent of metals precipitation/staining. 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.

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 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 three Muddy Creek mainstem stations were established by following a 40 x the average channel width methodology. Thus, a 300-m stream reach was electroshocked at mile point 0, a 300 m reach at mile point 2.1, and a 300 m reach at mile point 4.4. A 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. 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 the 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.

5.0 Results/Discussion

5.1 Habitat Evaluation

WAB personnel completed comprehensive RBP habitat surveys at each sample location using standard WAB protocol. In general, habitat quality across the three sample locations was similar and in reasonably good condition. 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, along with watershed scale disturbances.

<u>Epifaunal substrate/fish cover</u> is a category that expresses the quality of physical habitats in a stream in relation to fish and aquatic macroinvertebrates. In general, a high score in this category offers fish and benthic macroinvertebrates a substantial amount of diverse, quality habitats. There were no notable differences between pre- and post-treatment epifaunal substrate scores between any of the sample stations. Most sample

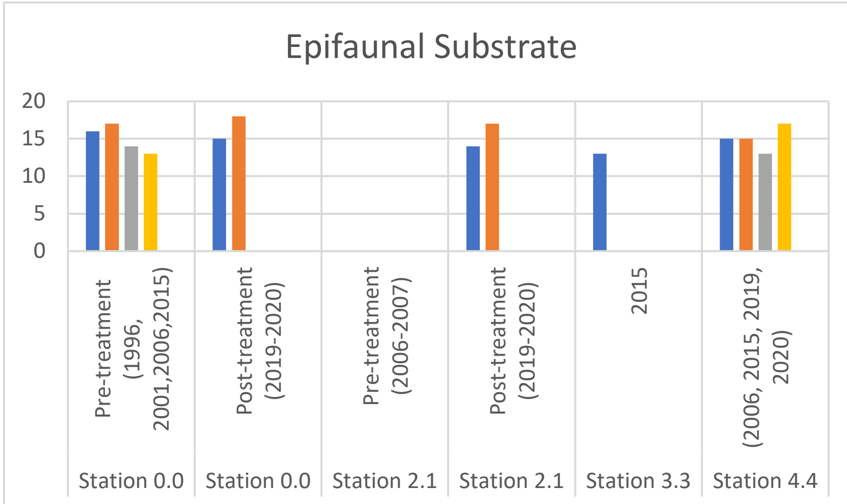


Figure 1- Pre- and post-treatment epifaunal substrate RBP ratings. Note: Multiple measurements taken across 2006 and 2007 due to monthly pre-TMDL monitoring.

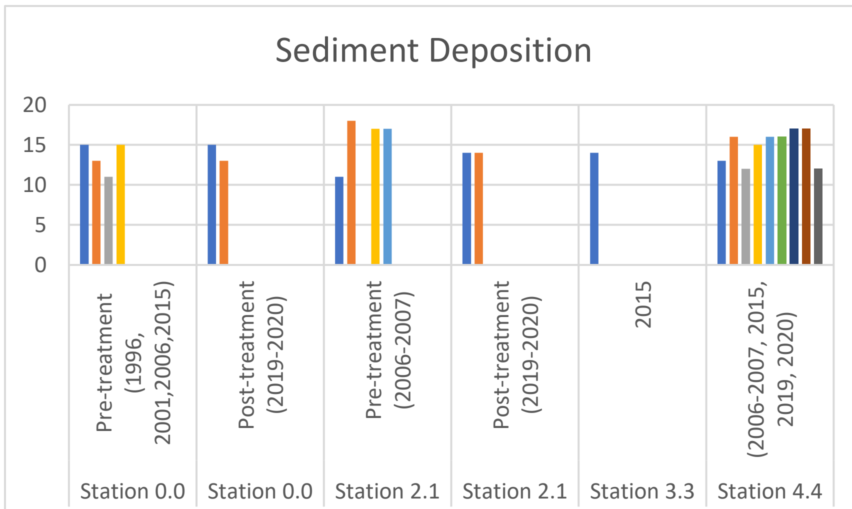


Figure 2- Pre- and post-treatment sediment deposition RBP ratings. Note: Multiple measurements taken across 2006 and 2007 due to monthly pre-TMDL monitoring.

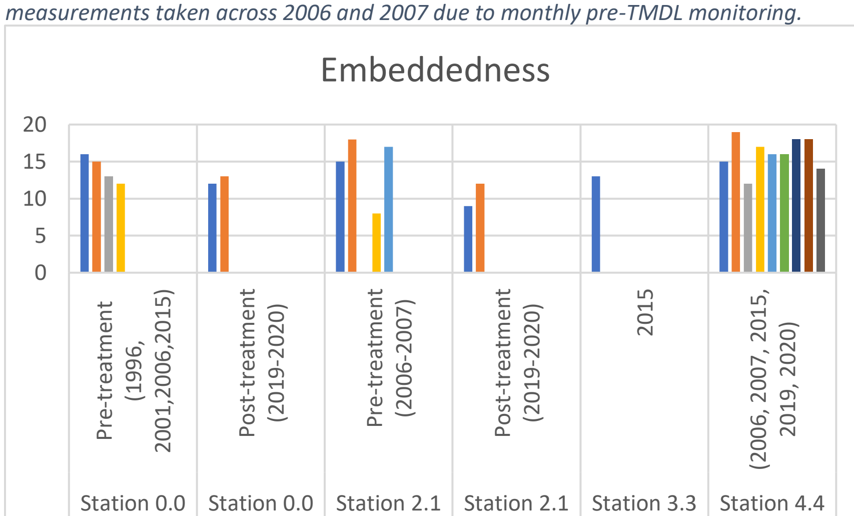


Figure 3- Pre- and post-treatment embeddedness RBP ratings. Note: Multiple measurements taken across 2006 and 2007 due to monthly pre-TMDL monitoring.

stations exhibited scores in the middle sub-optimal category, with a few scores in the optimal category (Figure 1).

The sediment deposition category 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. Pre- and post-treatment samples taken at all stations showed no notable differences between the two conditions. Most scores ranged from the middle to upper sub-optimal category into the optimal category (Figure 2).

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 available. In general, there were no substantial differences between pre-and post-treatment embeddedness scores. Although a few scores were in the marginal category, it is surmised that these lower scores could have been related to seasonal variability, or potentially . This category responds similarly to epifaunal and sediment deposition in terms of seasonal variability, flow dynamics, and watershed scale activities. Figure 3 shows a comparison of these scores.

5.2 Physio-chemical Samples

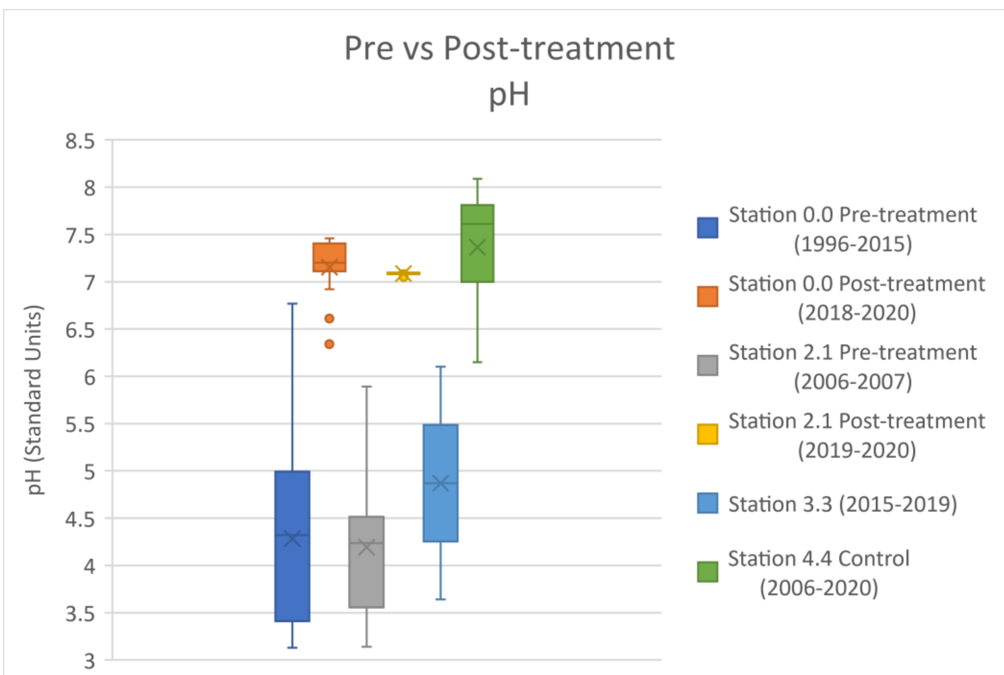


Figure 4- Pre- and post-treatment pH results.

WAB Staff collected 119 individual samples among the survey stations. These samples vary from simple field parameter discrete readings to comprehensive lab water quality analyses. It is important to note that there are fewer post-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 system.

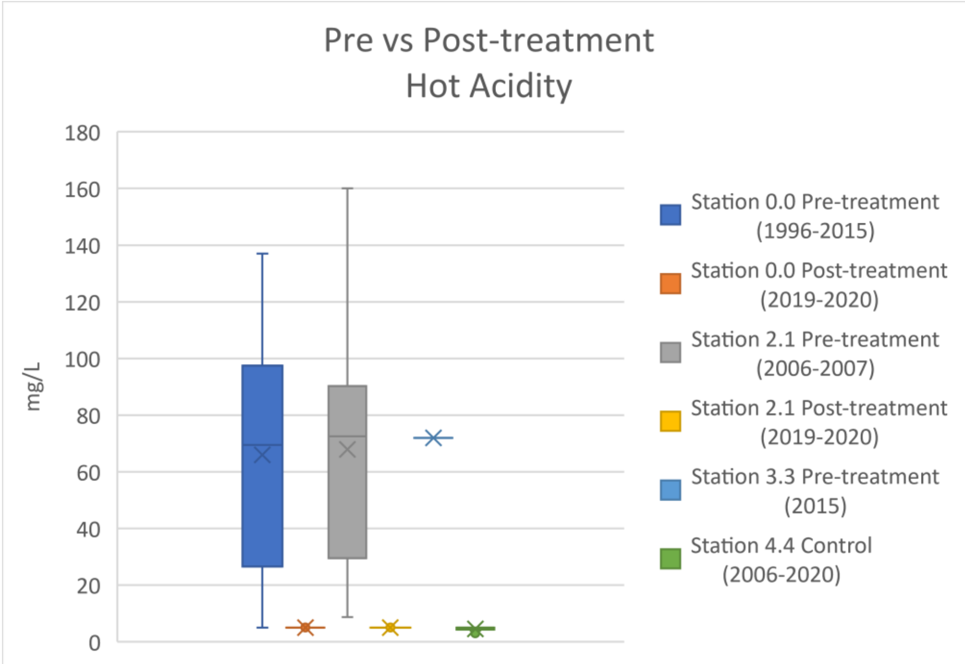


Figure 5- Pre- and post-treatment hot acidity results.

A significant improvement in pH has occurred at Stations 0.0 and 2.1 following the onset of treatment. Post-treatment pH values at Stations 0.0 and 2.1 (downstream of treatment) closely resemble Station 4.4 values. Median pre-treatment pH at Station 0.0 was 4.32. Station 2.1 showed a pre-treatment pH median of 4.24. Post-treatment pH medians at these stations were significantly higher. As of September of 2020, Station 0.0 and 2.1 median pH values were 7.2 and 7.09, respectively. In comparison, Station 4.4 pH median was 7.61 (Figure 4).

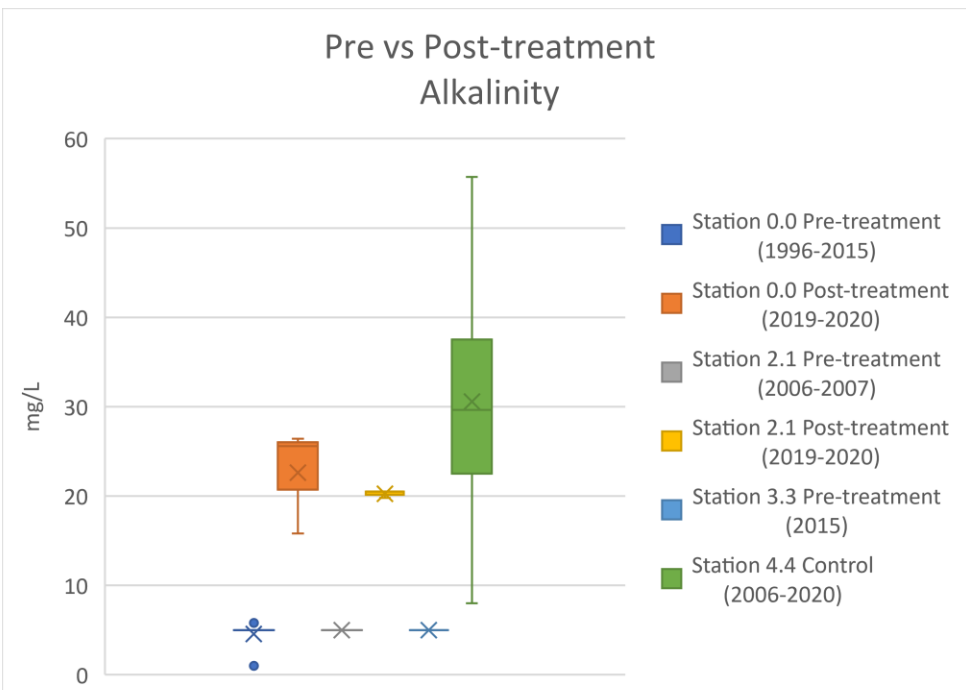


Figure 6-Pre- and post-treatment alkalinity results.

The deployable datalogger at mile 0.2 collected hourly pH reading to assess the effectiveness of treatment from the facilities upstream. Figure 14 in appendix B is a graph created using the hourly readings from March 2018, when treatment began, to January of 2019. During the first few months of treatment, pH was highly variable at times. This variability is likely due to treatment station calibration. After this calibration period, the graph shows steady fluctuations between pH 7.0 and pH 7.8.

In addition to the increase in pH, hot acidity concentrations were substantially reduced. Station 0.0 showed pre-treatment median concentrations of hot acidity to be 69.5 mg/L, with a maximum concentration of 137 mg/L collected during one sampling event. Station 2.1 showed very similar characteristics. Station 2.1 had a median concentration of 72.5 mg/L, with a maximum concentration of 160 mg/L collected. Post-treatment samples at these stations mirror conditions of Station 4.4. The minimum detection limit (MDL) of less than 5 mg/L have been achieved at Stations 0.0 and 2.1 (Figure 5).

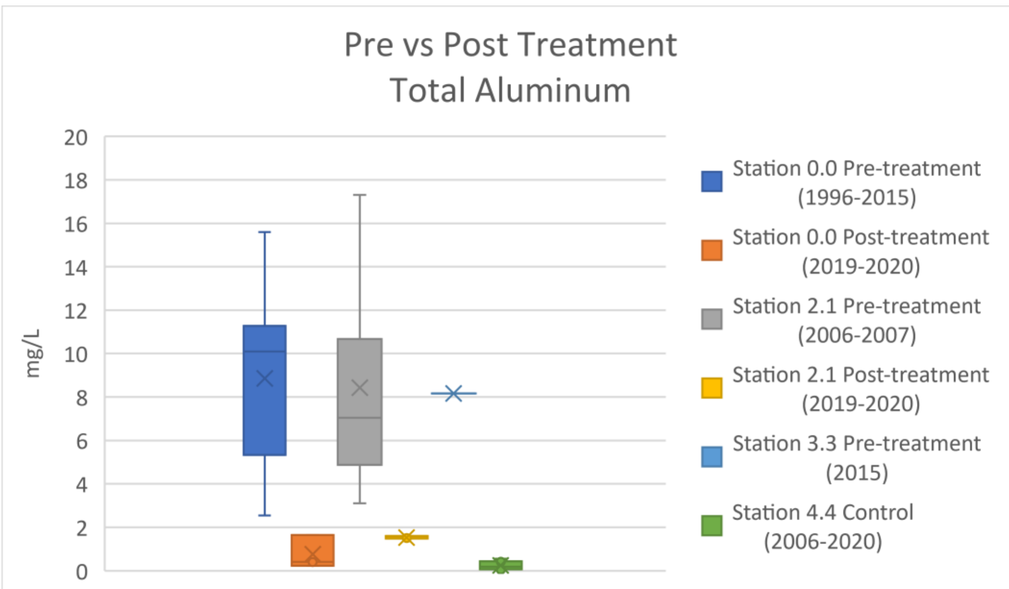


Figure 7- Pre- and post-treatment total aluminum results.

Alkalinity measures the amount of alkaline material in a water sample. It measures the stream's ability to neutralize acidic conditions. Pre-treatment conditions at Stations 0.0 and 2.1 showed concentrations of alkalinity at the MDL of <5 mg/L. In contrast, samples from station 4.4 had a median concentration of 28 mg/L. Post-treatment samples at Stations 0.0 and 2.1 were similar to Station 4.4. As of September 2020, median concentrations of 25.6 mg/L and 20.5 mg/L were collected from stations 0.0 and 2.1, respectively. Figure 6 is a box and whisker plot illustrating the differences between pre- and post-treatment data.

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. Aluminum specifically is highly toxic to aquatic organisms, but manganese and iron can have detrimental impacts as well.

Pre-treatment samples on Muddy Creek exhibited extremely high concentrations of aluminum. Station 0.0 had a median total aluminum concentration of 10.1 mg/L with a maximum of 15.6 mg/L. Station 2.1 had a median of 7.04 mg/L and maximum concentration of 17.3 mg/L. In contrast, station 4.4 had a median total aluminum concentration of 0.209 mg/L. Median total aluminum concentrations for over 700 samples of WAB Level I and II reference sites revealed a concentration of 0.09 mg/L. It is important to note the MDL for total aluminum is 0.02 mg/L. Although WAB has taken few comprehensive water samples at Stations 0.0 and 2.1 since the onset of treatment, post-treatment aluminum concentrations were significantly reduced. Three samples taken at Station 0.0 revealed concentrations of 0.4 mg/L, 1.64 mg/L and 0.231 mg/L. Figure 7 shows total aluminum concentrations among the sample stations.

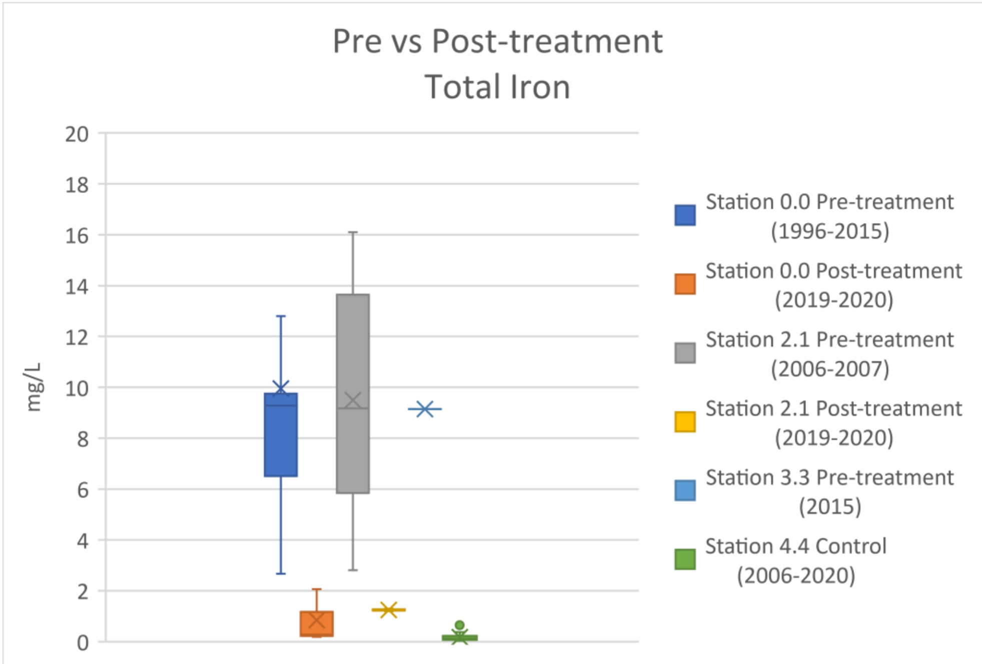


Figure 3- Pre- and post-treatment total iron results.

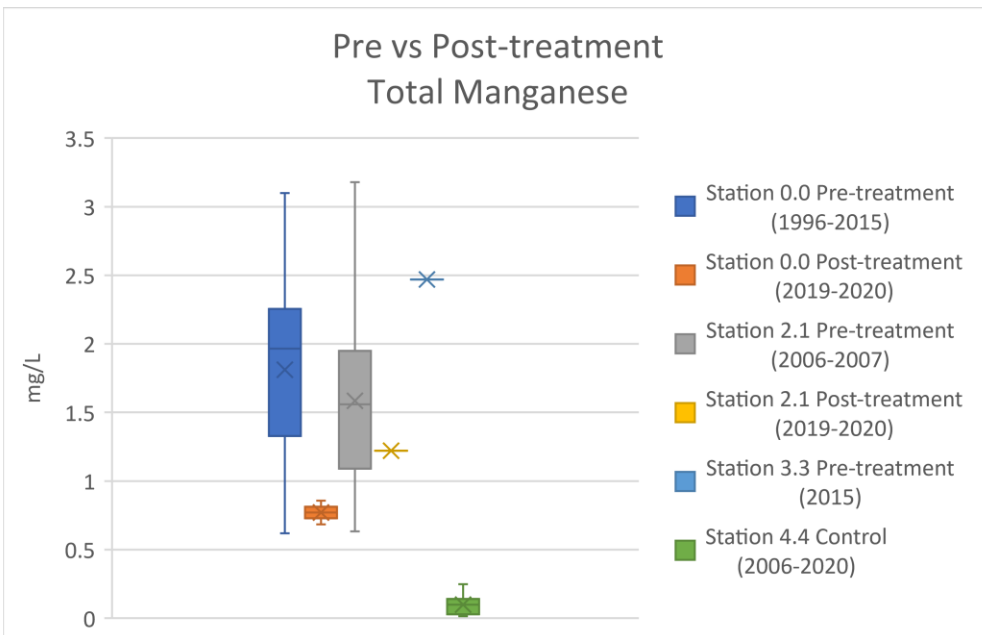


Figure 9- Pre- and post-treatment total manganese results.

It is important to note that concentrations of total metals, including aluminum, iron, and manganese, can exhibit natural variability due to stream flow and total suspended solids (TSS). Higher post-treatment values, like 1.64 mg/L, are likely caused by higher TSS situations. Metals such as iron, aluminum, and manganese 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 metals. This includes aluminum.

Sample Date	Mile Point	Fe Hydroxide Intensity	Al Hydroxide Intensity	Mn Hydroxide Intensity
6/21/2006	**0**	4	N/A	N/A
8/31/2006	**0**	3	N/A	N/A
9/27/2006	**0**	3	N/A	N/A
11/15/2006	**0**	4	N/A	0
12/5/2006	**0**	0	0	0
3/6/2007	**0**	4	0	0
5/29/2007	**0**	3	0	0
6/19/2007	**0**	4	0	0
9/17/2015	**0**	4	1	1
9/23/2019	**0**	2	1	0
9/16/2020	**0**	0	0	0
8/29/2006	**2.1**	4	N/A	N/A
9/25/2006	**2.1**	2	N/A	N/A
11/15/2006	**2.1**	4	N/A	2
12/5/2006	**2.1**	4	N/A	N/A
4/24/2007	**2.1**	3	0	0
5/29/2007	**2.1**	3	0	0
6/19/2007	**2.1**	4	0	0
9/24/2019	**2.1**	3	1	2
9/16/2020	**2.1**	3	1	1
9/16/2015	**3.3**	3	3	2
6/21/2006	**4.4**	N/A	N/A	1
8/31/2006	**4.4**	N/A	N/A	1
9/27/2006	**4.4**	N/A	N/A	2
10/17/2006	**4.4**	0	0	0
11/15/2006	**4.4**	0	0	2
12/6/2006	**4.4**	0	0	3
1/10/2007	**4.4**	0	0	2
2/7/2007	**4.4**	0	0	3
3/6/2007	**4.4**	0	0	0
4/24/2007	**4.4**	0	0	0
5/30/2007	**4.4**	0	0	0
6/19/2007	**4.4**	0	0	1
9/16/2015	**4.4**	0	0	0
9/24/2019	**4.4**	0	0	0
9/15/2020	**4.4**	0	0	0

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

Total iron was significantly reduced at Stations 0.0 and 2.1 following the onset of treatment. Pre-treatment medians for Stations 0.0 and 2.1 were 9.28 mg/L and 9.17 mg/L, respectively. Three post-treatment samples taken at Station 0.0 exhibited concentrations of 0.27 mg/L, 2.06 mg/L and 0.18 mg/L. Station 2.1 had three post-treatment samples taken with concentrations of 1.25 mg/L, 1.2 mg/L, and 1.3 mg/L. In contrast, Station 4.4 had a median concentration of 0.109 mg/L (Figure 8).

Similar to aluminum and iron, total manganese concentrations were also reduced. Pre-treatment samples at Stations 0.0 and 2.1 had median concentrations of 1.97 mg/L and 1.56 mg/L, respectively. After treatment, two samples from Station 0.0 had values of 0.685 mg/L and 0.856 mg/L. Station 2.1 had a post-treatment concentration of 1.25 mg/L. Station 4.4 had a sample median of 0.099 mg/L (Figure 9).

5.3 Metal Precipitates

Muddy Creek Precipitate Analysis Station 0.0			
Parameter	**Value**	**Units**	**MDL**
Total Arsenic	*< 0.18*	*mg/kg*	*0.18*
Total Barium	*5.12*	*mg/kg*	*0.08*
Total Cadmium	*0.49*	*mg/kg*	*0.05*
Total Chromium	*0.73*	*mg/kg*	*0.15*
Total Lead	*0.15*	*mg/kg*	*0.13*
Total Mercury	*< 0.12*	*mg/kg*	*0.005*
Total Selenium	*< 0.10*	*mg/kg*	*0.1*
Total Silver	*< 0.05*	*mg/kg*	*0.05*
Total Aluminum	*4366*	*mg/kg*	*0.23*
Total Iron	*2927*	*mg/kg*	*0.1*
Total Manganese	*429*	*mg/kg*	*0.18*

Table 2-Precipitate analysis from Station 0.0.

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.

In general, visual metal hydroxide intensity ratings at Station 0.0 were somewhat reduced following the start of treatment. In 2006-2007 and 2015, pre-treatment data from this station had iron hydroxide ratings of 3 and 4. After treatment, that rating had reduced to a 2. Station 2.1 showed no notable differences between pre- and post-treatment samples. It is important to note that many years of AMD have stained the substrate in Muddy Creek so extensively that it likely will always show signs of metals deposition, especially iron. The initial results at Station 0.0 are positive, however. In September of 2020, visual metal hydroxide intensity ratings at station 0.0 were rated zeros for iron, aluminum, and manganese. WAB staff noted that remnant iron and aluminum staining were still visible, however. Table 1 shows metal hydroxide intensities from the sample stations.

In 2019, WAB and OSR staff observed a precipitate formation on the substrate at Station 0.0. This precipitate is commonly found in treated systems and usually consists of metals, periphyton/algae, and sediments. A sample was taken by OSR staff and analyzed in a lab. Total aluminum, iron, and manganese concentrations were extremely high. A total aluminum concentration of 4,366 mg/kg was reported. Total iron and total manganese concentrations were 2,927 mg/kg and 429 mg/kg, respectively. Table 2 shows results from the sediment sample taken at Station 0.0. It is important to note the MDLs for these constituents: aluminum is 0.23 mg/kg, iron is 0.1 mg/kg, and manganese is 0.18 mg/kg.

In September of 2020, WAB staff noted that precipitate formation was still prevalent at station 0.0. Samples were not taken in 2020. It is likely that similarities would exist between what was seen in 2020 with what was sampled in 2019.

5.4 Benthic Macroinvertebrate Sampling

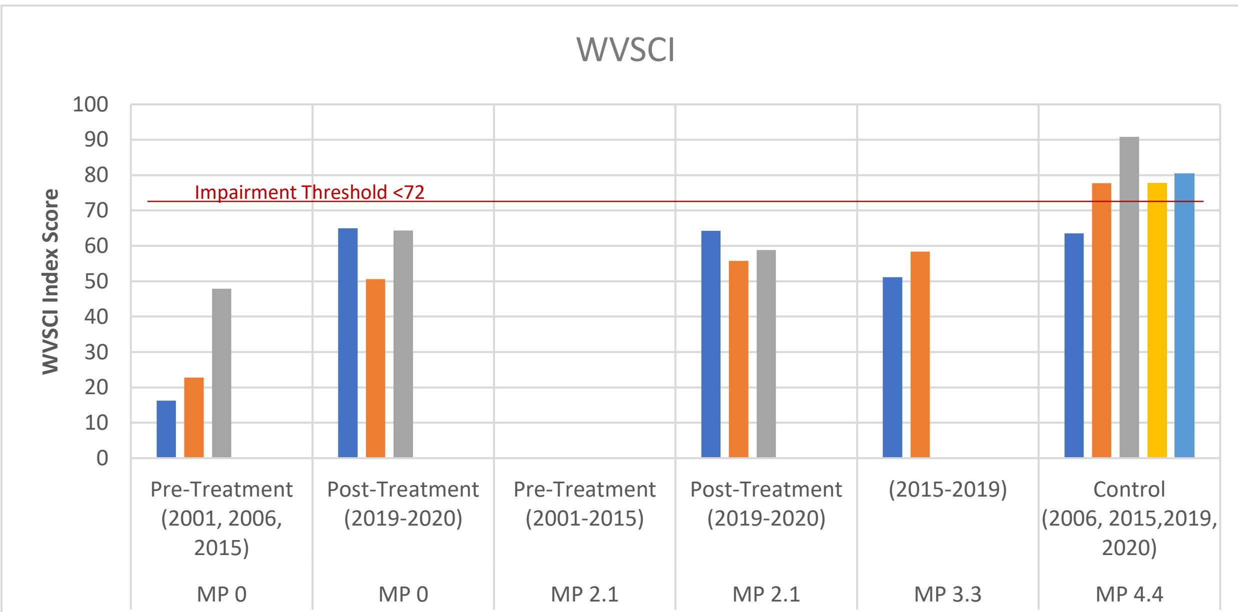


Figure 10- Pre-and post-treatment WVSCI scores. Note: Two samples were collected in 2020.

Sixteen individual benthic macroinvertebrate samples were collected between the sample stations from 2001 to 2020. Similar to other parts of the study, data from previous samples are included in this report to boost the dataset. It is important to note that no pre-treatment benthic samples from Station 2.1 exist due to WAB personnel establishing this site for biological monitoring in 2019. Also, two samples were taken in 2020. One sample was taken in June and the other in September.

WAB uses the West Virginia Stream Condition Index (WVSCI) to assess the biological condition of streams. The WVSCI summarizes family level identifications of benthic macroinvertebrate assemblages to assess the biological condition of wadeable streams with riffle/run habitats. This index includes six biological metrics that represent elements of the structure and composition of benthic macroinvertebrate communities. Because larval macroinvertebrates are relatively stationary, they are 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 families.

At Station 0.0, the WVSCI score improved from a pre-treatment median score of 22.79 to a post-treatment score of 64.95 in 2019. In June of 2020, the WVSCI score at station 0.0 was 64.38. The September sample was 50.62. The impairment threshold for WVSCI is 72. This means that any score less than 72 is considered impaired. Sites scoring over 72 are considered unimpaired. Lower scores, like 22.79 for example, are considered severely impaired. Station 2.1 had a WVSCI score of 64.29 in 2019. The 2020 samples from June and September yielded WVSCI scores of 58.84 and 55.77, respectively. The sample median WVSCI score for Station 4.4 was 83.31 (Figure 10). It is important to note that pre-treatment WVSCI scores from Stations 0.0, 3.3 and post-treatment scores from Station 2.1 in 2019 and June of 2020 had macroinvertebrate densities below 100 organisms/square meter. The WVSCI requires samples

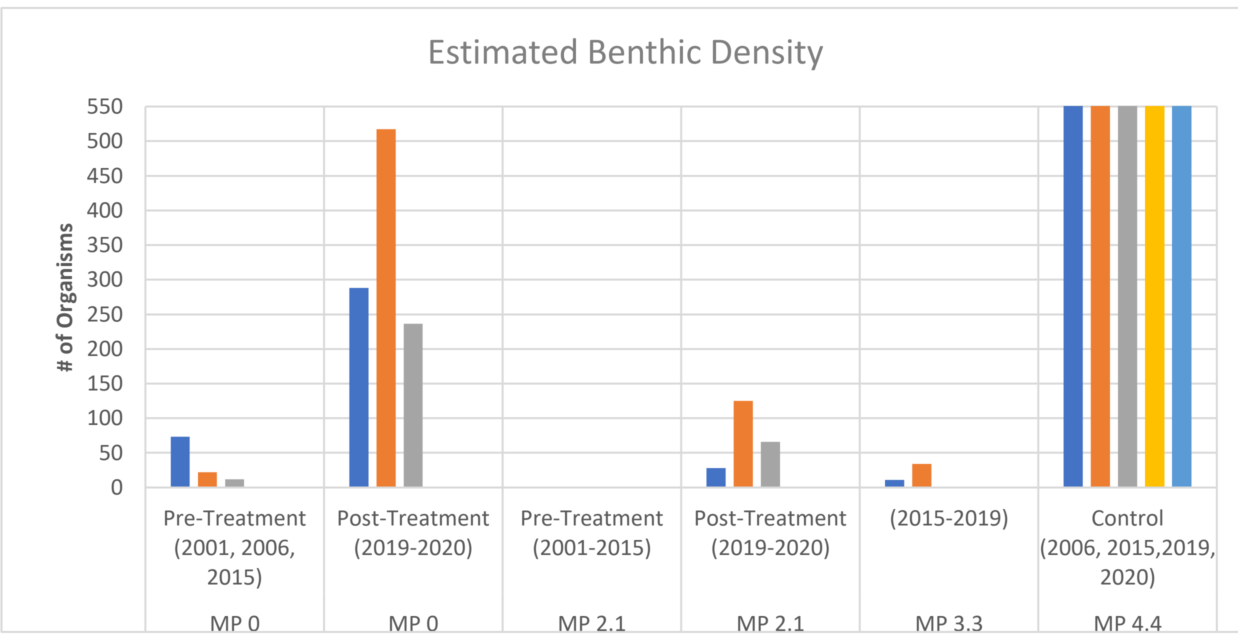


Figure 11- Pre-and post-treatment estimated benthic densities. Note: Two samples were collected in 2020 and Station 4.4 densities are much higher than what is shown.

to have at least 100 organisms to provide an accurate score.

Benthic Density is an important component when assessing treated stream systems. In these conditions, benthic densities are often significantly diminished. The WAB can estimate benthic macroinvertebrate density via an extrapolation technique from the area sampled in the stream. Density values indicate how many organisms per square meter are estimated to be present

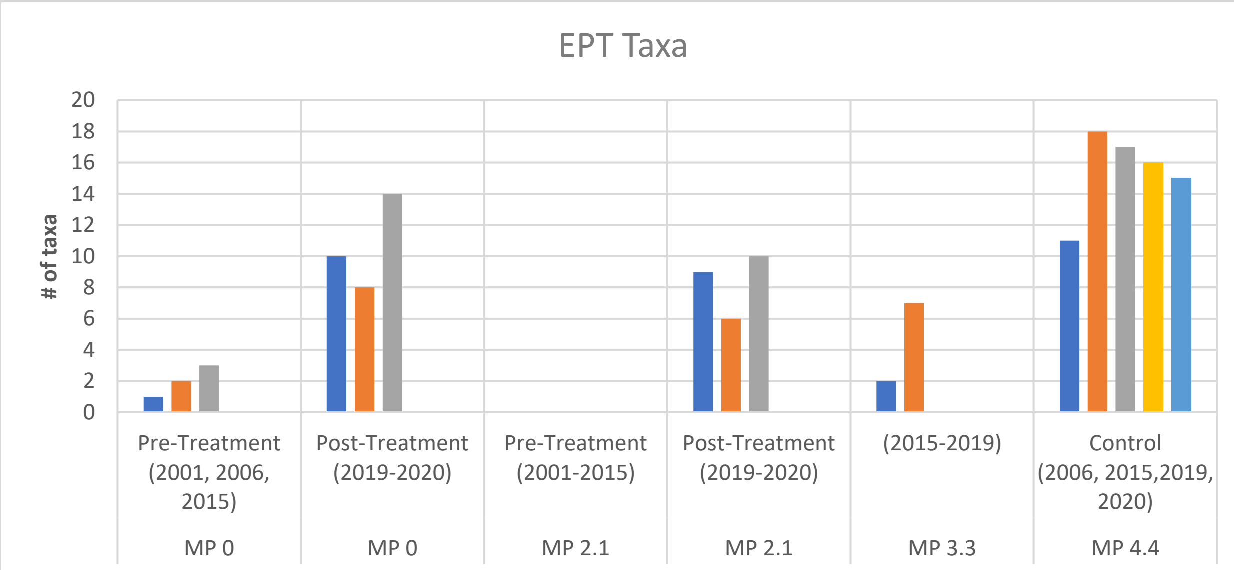


Figure 12- Pre-and post-treatment EPT taxa richness. Note: Two samples were taken in 2020.

within the stream at a location. Looking further into available data showed some notable information about Stations 0.0 and 2.1. Pre-treatment samples at Station 0.0 and post-treatment samples at Station 2.1 in 2019 and June of 2020 revealed that benthic density was very low. These samples displayed benthic densities of less than 100 individual organisms. When densities are this low, the entire sample is picked. At Station 0.0, three samples taken between 2001 and 2015 exhibited densities of 73 organisms/m², 22 org/m², and 12 org/m². The post-treatment sample at Station 0.0 showed positive results. Benthic density estimates rose to 288 org/m². Post-treatment samples at Station 2.1 in 2019 and June of 2020 did not show positive results, however. Only 28 total organisms were collected in the sample from 2019 and 62 organisms in June of 2020. On a positive note, the sample from September of 2020 collected a total of 124 organisms. For comparison purposes, Station 4.4 had a median benthic density estimate of 3,316 org/m² (Figure 11). The figure does not show the upper limits of estimated density for this station.

One possible explanation for the low density of organisms at Station 2.1 could be metals deposition. The previous impacts of AMD on the substrate and hyporheic zone were extremely detrimental to benthic macroinvertebrate substrate. Many years of AMD and metal precipitates likely still reside within these areas and can continue to be uninhabitable for benthic organisms. Along with these legacy impacts, the depositing flocculants from current treatment processes can negatively impact benthic communities as well. This deposition contains high concentrations of aluminum, iron, and manganese which can be detrimental to benthic organisms. Another important factor to consider when determining the effectiveness of AMD treatment is the number of Ephemeroptera, Plecoptera, and Trichoptera (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 fewer EPT taxa than higher quality streams. Pre-treatment samples from Station 0.0 showed EPT taxa richness to be very low. Pre-treatment samples at this station revealed a median EPT taxa richness of 2. The post-treatment sample taken in 2019 showed a significant increase in EPT taxa richness, going from 2 taxa to 10 taxa. The samples from June and September of 2020 yielded 14 and 8 EPT taxa, respectively. Nine EPT taxa were identified at Station 2.1 in 2019. Samples from June and September of 2020 collected 10 and 6 EPT, respectively. It is important to note that seasonal variation can exist between samples. Benthic community compositions often change throughout the year due to life history strategies of organisms. This could be one possible explanation in reduced EPT taxa abundance between the June and September 2020 samples. In

Muddy Creek Fish Community Comparison Pre-treatment (2015) vs Post-treatment (2019-2020)										
Mile Point	**0**			**2.1**			**3.3**	**4.4**		
Sample Year	**2015**	**2019**	**2020**	**2015**	**2019**	**2020**	**2015**	**2015**	**2019**	**2020**
River Chub		111								
Smallmouth Bass		12								
Rock Bass		2								
Rosyside Dace		1								
Green Sunfish		3			12					
Mottled Sculpin		1			3			225	653	
Spotfin Shiner	No Fish Collected	1	Did Not Survey	Did Not Survey		Did Not Survey	No Fish Collected			Did Not Survey
Stonecat		2								
Rosyface Shiner		10								
Creek Chub					10			301	191	
Western Blacknose Dace								461	485	
White Sucker								22	82	
Longnose Dace								26	27	
Brown Trout								6	1	
Rainbow Trout					1				2	
Total Species	**0**	**9**	**0**	**0**	**4**	**0**	**0**	**6**	**7**	**0**
Total Collected	**0**	**143**	**0**	**0**	**26**	**0**	**0**	**1041**	**1441**	**0**
Fish/meter	**0.00**	**0.48**	**0.00**	**0.00**	**0.09**	**0.00**	**0.00**	**3.79**	**5.24**	**0.00**

Table 3-Pre- and post-treatment fish community survey results.

comparison, Station 4.4 contained a median EPT taxa richness of 17 (Figure 12).

5.5 Fish Community Sampling

Six fish community surveys have been completed among the Muddy Creek sample stations. Two surveys at Station 0.0, one survey at Station 2.1, one survey at Station 3.3, and two surveys at Station 4.4. A comparable pre-treatment survey was not taken at Station 2.1 and a post-treatment survey was not taken at Station 3.3 due to low visibility within the water column.

Pre-treatment surveys conducted in 2015 at mile 0.0 and 3.3 yielded no fish due to extensive AMD impacts. After treatment began, water quality conditions became more favorable for fish passage into the Muddy Creek mainstem. In 2019, the fish community survey at Station 0.0 yielded 143 individual fish comprised of 9 unique species. The fish community survey at Station 2.1 yielded only 26 individual fish comprised of 4 unique species. In comparison, Station 4.4 yielded 1,041 individual fish of 6 different species in 2015. In 2019, the survey collected 1,441 individuals of seven different species. It is important to note two interesting occurrences during the 2019 surveys at 0.0 and 2.1. Mottled sculpins, a benthic species, were collected at each of these stations. One individual at 0.0 and three at Station 2.1. Mottled sculpin prefer cool and cold-water systems and are considered to be moderately sensitive to pollution. Collecting this species in the lower reaches of Muddy Creek is a positive sign that conditions are improving. Another sign of improvement is the presence of trout in Muddy Creek. The survey at Station 2.1 yielded one rainbow trout in 2019. Like mottled sculpin, rainbow trout occupy cool and cold-water streams and are moderately sensitive to pollution and temperature. It is remarkable that trout are now able to utilize the mainstem of lower Muddy Creek, albeit in low numbers thus far. Trout were present during the surveys of Station 4.4 as well (Table 3).

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 fish/m scores at Stations 0.0 and 3.3 were zero because no fish were collected. In 2019, post-treatment surveys of Stations 0.0 and 2.1 yielded metric scores of 0.48 and 0.09, respectively. Station 4.4 results were much higher. In 2015, Station 4.4 had a metric score of 3.71 fish/m while 2019 scores were even higher with 5.24 fish/m. Stations 0.0 and 2.1 did not have high metric scores compared to Station 4.4, but positive outcomes were achieved, nonetheless.

Unfortunately, additional fish surveys planned for 2020 were unable to be completed due to COVID-19 restrictions. WAB staff plan to complete these surveys in 2021.

6.0 Conclusions

Based on the findings of this study, the conditions in the lower reaches of Muddy Creek have <u>improved substantially</u> since the onset of AMD treatment. Notable increases in physiochemical properties like pH and alkalinity coupled with decreases in total and dissolved metals have been observed. Continuous water quality monitoring showed increases in hourly pH readings as well as sustainability of those pH values throughout the year of treatment.

Stream RBP habitat conditions were found to be mostly favorable and usually remained in the high sub-optimal and optimal categories. Although some variability exists within the sample stations, habitat conditions are generally favorable for aquatic organisms and are likely not limiting biological recovery to a large degree. Flocculant deposition downstream of the treatment facility may be limiting the benthic macroinvertebrate recovery at some level, however.

Benthic macroinvertebrate samples collected at the sample stations showed mixed results. Station 0.0 showed noticeable increases in WVSCI score, estimated density, and # of EPT taxa. Results from Station 2.1 trended in the positive direction. Estimated benthic density rose to 66 org/m^2 in June of 2020 and again in September of 2020 to 125 org/m^2. The highest number of EPT taxa, 10, were collected at this station in June as well. While some improvements were seen, recovery of the benthic community has not been substantial at this station yet.

The fish community response to AMD treatment was positive. No fish were collected in the mainstem of Muddy Creek at any station downstream of Martin Creek prior to treatment – the stream was essentially devoid of fish. Post-treatment surveys revealed an increase in species richness and abundance, most notably at Station 0.0. This station's close proximity to the Cheat River mainstem will provide good fish recruitment potential for the lower reaches of Muddy Creek. Although Station 2.1 did not exhibit high species richness or abundance, it did have a positive outcome. Mottled sculpin and rainbow trout were collected at this station. Seeing these moderately sensitive fish species returning to the lower reaches of Muddy Creek is very significant. The fish community at Station 4.4 is very well established based on WAB survey data. This section has great potential to help recruitment in Muddy Creek. Over time, it is expected that fish populations from this section of Muddy Creek will emigrate downstream and populate stream reaches below Martin Creek.

This project is an ongoing study and will require more surveys and data to be collected to fully assess the biological recovery of Muddy Creek. WAB and OSR staff will continue to monitor and track changes within Muddy Creek in the future and provide insight into the effectiveness of treatment on the biological community.

Appendix A

Muddy Creek Sample Station Locations		
Station	**Latitude**	**Longitude**
Mile 0	*39 ° 30' 41.32"*	*79 ° 38' 51.2"*
Mile 0.2	*39 ° 30' 45.7"*	*79 ° 38' 47.3"*
Mile 2.1	*39 ° 32' 4.9"*	*79 ° 37' 54.7"*
Mile 3.3	*39 ° 32' 59.3"*	*79 ° 37' 53.0"*
Mile 4.4	*39 ° 32' 36.0"*	*79 ° 37' 26.2"*

Table 5-Sample station locations.

Muddy Creek Treatment Stations		
Facility	**Latitude**	**Longitude**
T&T Treatment Facility	*39 ° 32' 37.09"*	*79 ° 37' 49.71 "*
Glade Run Treatment Facility	*39 ° 33' 7.29"*	*79 ° 39' 7.10 "*

Table 4-Treatment station locations.

Appendix B

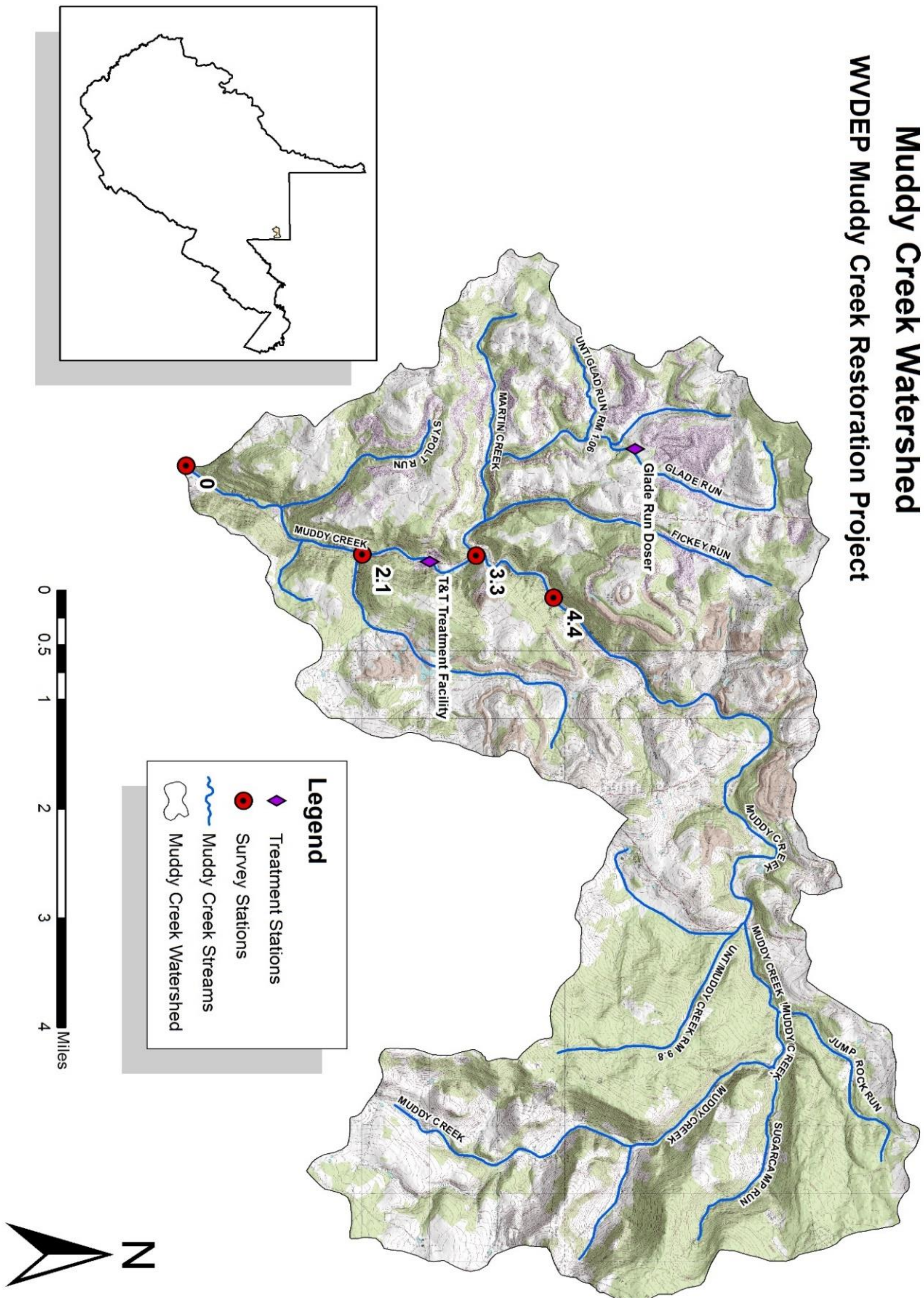


Figure 13-Map of the study area.

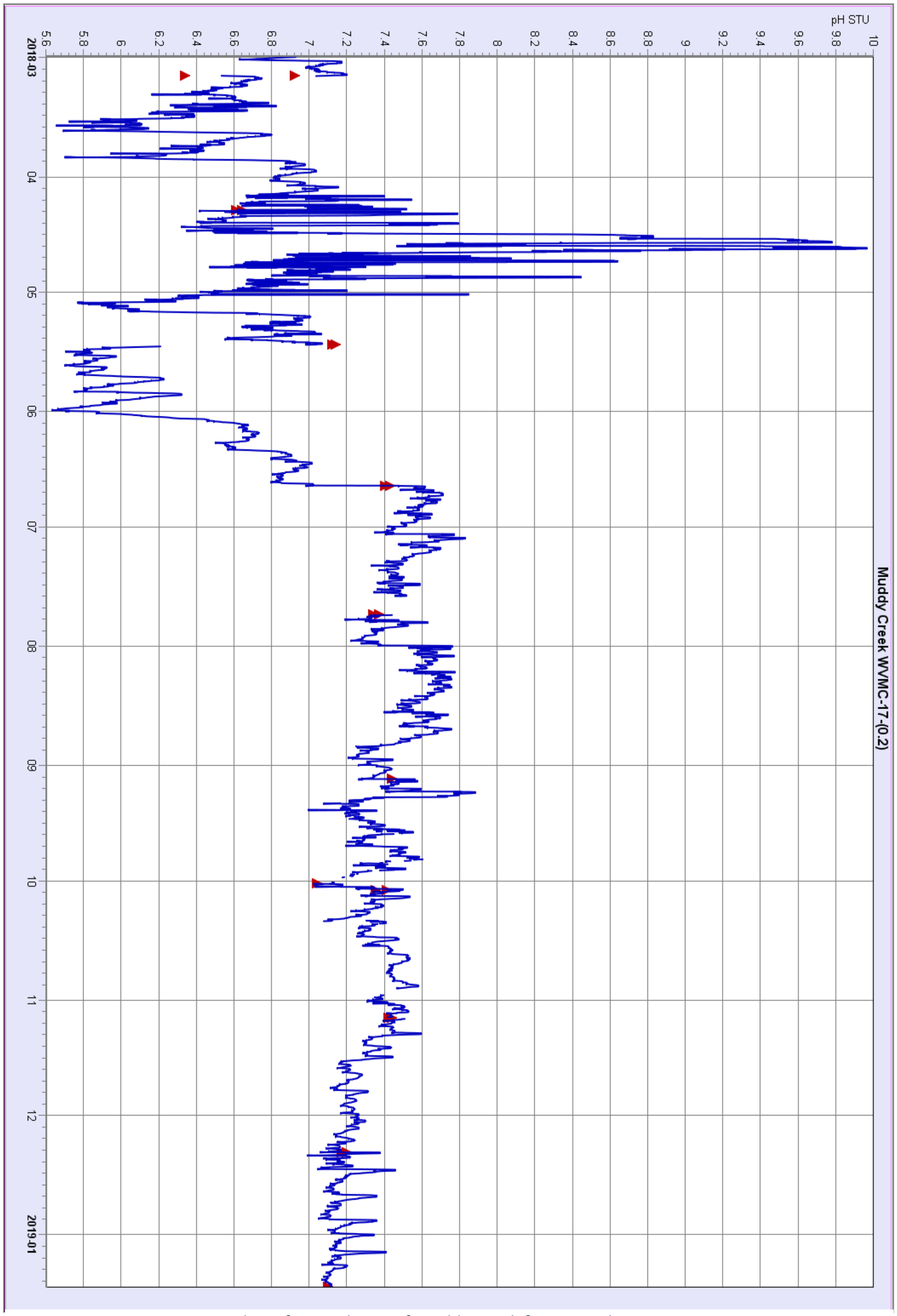


Figure 14- Continuous pH readings from mile 0.2 of Muddy Creek from March 2018 to January 2019.

Appendix C

Figure 15-Looking upstream from Station 0.0 in 2015.

Figure 16- Looking upstream from Station 0.0 in 2019.

Figure 17-Precipitate mix at Station 0.0.

Figure 18-Looking upstream from Station 2.1

Figure 19-Rainbow Trout collected at Station 2.1 during the 2019 electrofishing survey.

Figure 20-Confluence of Martin Creek and Muddy Creek in 2015.

Figure 21-Looking upstream of station 3.3 in 2015.

Figure 22-Looking upstream from Station 4.4.

Figure 23-Brown Trout collected from electrofishing survey at Station 4.4.