Monitoring Biological Recovery in the Twomile Run Watershed Following Abandoned Mine Drainage Remediation Clinton County, Pennsylvania

November 2013

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Objectives

- Document and monitor biological recovery (both benthic macroinvertebrates and fish populations) following the treatment of several abandoned mine drainage (AMD) pollution sources in the Twomile Run watershed.
- Determine the length of time necessary for previously impaired reaches to recover to a point comparable to unimpaired reaches.
- Using morphometric data, determine if there differences exist in the health or condition of brook trout inhabiting previously impaired reaches compared to unimpaired and control reaches.
- As brook trout begin to recolonize new areas of the Twomile Run watershed, document the source populations of the fish through movement studies and genetics. These data will also confirm that previously isolated populations are interbreeding.

Methods

A total of 14 sample sites were identified by Trout Unlimited throughout the Twomile Run watershed in order to monitor biological recovery in the watershed as a result of AMD treatment (Figure 1). Table 1 details the sample site locations and provides a description for each sample site. These sites were established for long-term monitoring and will be used for benthic macroinvertebrate collections and fishery surveys.

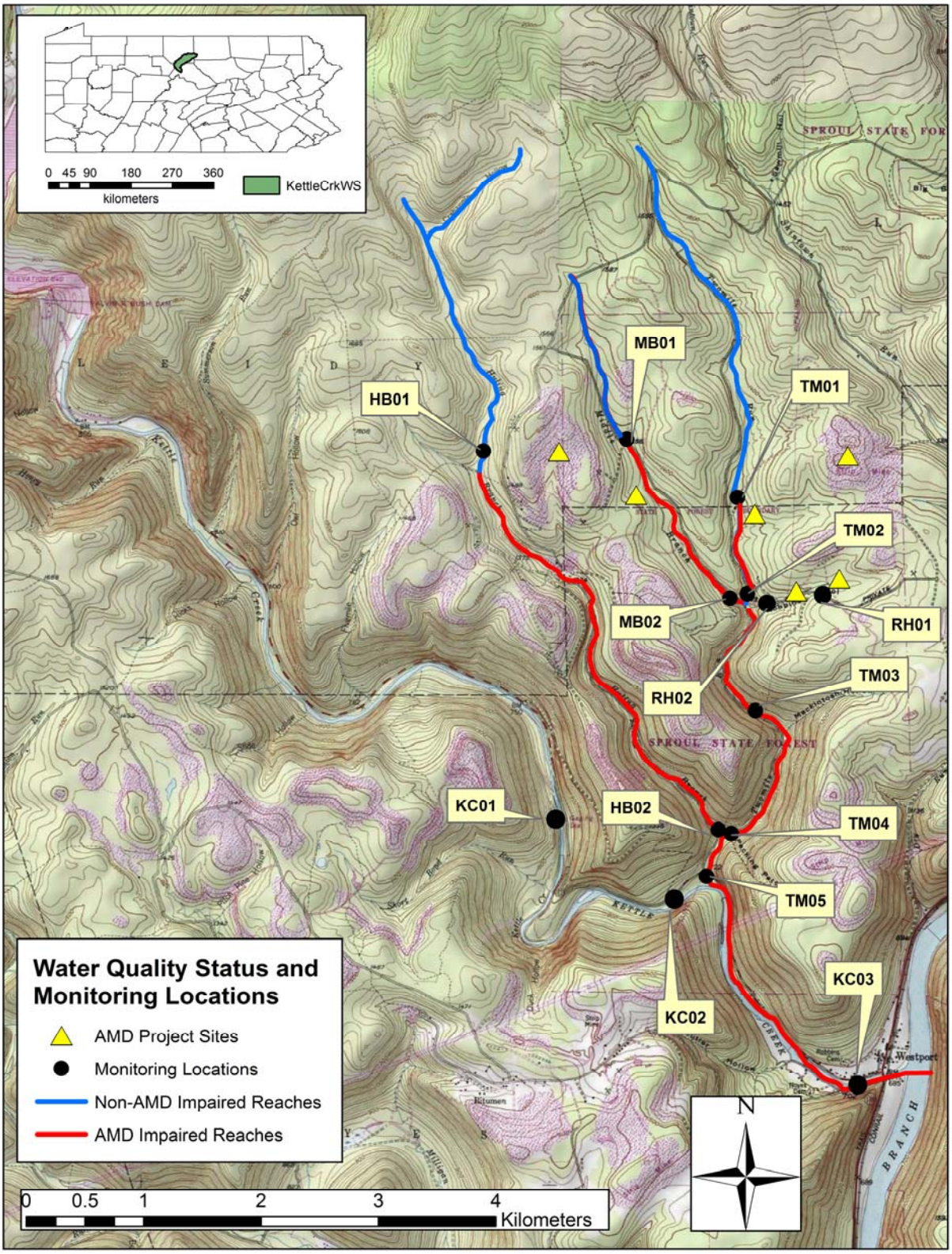


Figure 1: Map of Twomile Run watershed with monitoring and AMD project locations.

Table 1: Twomile Run watershed sample site descriptions and locations.

Name	Description	Lat	Lon
TM01	Twomile upstream of unnamed trib	41.344645	-77.856308
TM02	Twomile below unnamed trib	41.33727	-77.8552
TM03	Twomile below Robbins Hollow; above Mackintosh Hollow & alkaline discharge	41.328322	-77.85426
TM04	Twomile below Mackintosh Hollow & alkaline discharge	41.318873	-77.856602
TM05	Twomile below Huling	41.315568	-77.859087
RH01	Robbins Hollow below North and East branch juncture	41.337724	-77.849493
RH02	Robbins Hollow near mouth	41.336475	-77.85431
MB01	Middle Branch above treatment	41.349037	-77.867625
MB02	Middle Branch below treatment	41.336923	-77.856966
HB01	Huling Branch near walking bridge	41.348021	-77.882223
HB02	Huling Branch below remining / reclamation	41.319149	-77.857933
KC01	Kettle Creek above AMD impacts	41.319608	-77.873954
KC02	Kettle Creek upstream of Twomile	41.314178	-77.860907
KC03	Kettle Creek at Westport	41.299747	-77.843273

Benthic Macroinvertebrate Communities

Benthic macroinvertebrate sampling began in 2008 on Middle Branch, the first tributary to Twomile Run to under restoration from the Middle Branch passive treatment system. Benthic macroinvertebrates were collected in compliance with PA Department of Environmental Protection (DEP) protocol (see below). All benthic macroinvertebrate collections were made according to the DEP Instream Comprehensive Evaluation (ICE) protocol (specifically section C.1.b. *Antidegradation Surveys*). In short, benthic macroinvertebrate samples consisted of a combination of six D-frame efforts in a 100-meter stream section. These efforts were spread out to select the best riffle habitat areas with varying depths. Each effort consisted of an area of 1 m^2 to a depth of at least 4 inches as substrate allowed and was conducted with a 500 micron mesh 12-inch diameter D-frame kick net. The six individual efforts were composited and preserved with ethanol for processing in the laboratory.

Individuals were identified by taxonomists certified by the North American Benthological Society to genus or to the next highest possible taxonomic level. Samples containing 160 to 240 individuals were evaluated according to the six metrics comprising the DEP's Index of Biological Integrity (IBI) (Total Taxa Richness, EPT Taxa Richness, Beck's Index V.3, Shannon Diversity, Hilsenhoff Biotic Index, and Percent Sensitive Individuals). Appendix A contains a description of each of these six metrics. These metrics were standardized and used to determine if the stream met the Aquatic Life Use (ALU) threshold for coldwater fishes, warmwater fishes, and trout stocked fishes (Figure 2). Biological metrics are provided for sites containing less than 160 individuals however, an IBI score was not calculated for these sites because sites with less

than 160 individuals do not qualify according to DEP criteria. Benthic macroinvertebrates will continue to be monitored on an annual basis as funding allows.

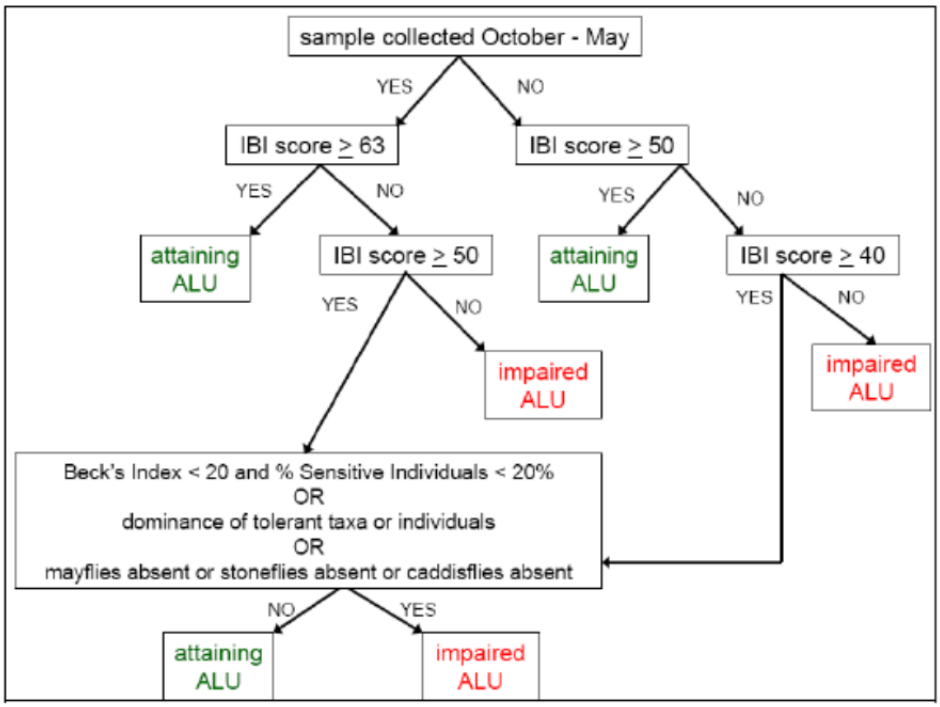


Figure 2: ALU Attainment and Impairment Thresholds for Coldwater Fishes (CWF), Warmwater Fishes (WWF), and Trout Stocked Fishes (TSF) Protected Uses (PA Department of Environmental Protection, 2009).

Fishery Surveys

Fishery surveys began in 2008 on Middle Branch, the first tributary to Twomile Run to under restoration from the Middle Branch passive treatment system. Surveys were completed using a Smith-Root, model LR-24 backpack electrofisher during summer low-flow conditions to minimize sampling bias and allow for the capture of young-of-year fish. Each survey proceeded upstream for a minimum of 100 meters. At sites containing trout, three-pass removal/depletion methodology was used. Only a single electrofishing pass was completed at sites where no trout were captured. Proper current and voltage settings were determined on-site following an evaluation of conductivity. Sample sites located on the mainstem of Kettle Creek were surveyed by the Pennsylvania Fish and Boat Commission using a towboat electrofisher due to the width and depth of the stream at these locations. All fish captured during the electrofishing surveys were identified to species. Brook trout that were collected were measured for length to the nearest millimeter and separated into 25 mm size classes. Density was also calculated from these data.

Brook Trout Morphometrics

In addition to total length measurements, brook trout collected from sample sites HB01, MB01, MB02, and TM01 were utilized for morphometric analysis. Trout Unlimited worked in collaboration with Lock Haven University. In addition, trout were also collected from the Larrys Creek watershed (Lycoming County) and were used as a control population (i.e. the absence of AMD). In total 233 brook trout were included in the morphometric analysis. Brook trout from the Twomile Run watershed were grouped into two groups, 63 trout from above sever AMD impact and 100 trout below AMD treatment (MB02). Based on the severely degraded water quality, the trout collected above the AMD seep have been isolated from downstream locations for more than 50 years. The trout collected below AMD seepages were living in habitat with mildly degraded water quality. The 70 trout from Larrys Creek watershed were collected from numerous streams, representing an interconnected population.

In all locations, trout were collected using standard electrofishing procedures by Lock Haven University students, Trout Unlimited, and the Pennsylvania Fish and Boat Commission. After normal counting/measuring or the population survey, each trout was lightly anesthetized and photographed with dorsal, pelvic, and anal fins extended (Figure 3). Care was taken to position the fish straight on the photograph board. We choose fish from as many age classes as possible with the limitation that photographing smaller fish is difficult; therefore age 0 and age 1 fish are excluded.

The freeware TPSDig (Rohlf 2005a) was used to digitize landmarks at 13 easily identifiable positions on the body of the fish (i.e. fin insertions, edge of gill cover, etc.). TPS RelWarp (Rohlf 2005b) was then used to create a consensus configuration for the entire sample. The consensus configuration is equivalent to the average X, Y position of each landmark after scaling each fish to the same size (Figure 4).

Finally, relative warp scores were calculated for the sample using TPS RelWarp (Rohlf 2005b). Relative warps are analogous to principal components in that they summarize correlated variation onto the same factor. The advantage of using relative warps analysis is that the correlated variation summarized on a specific warp can be visualized using thin plate spline diagrams. Thin plate splines visually represent the differences from the consensus configuration along specific relative warps. To explore possible patterns in body shape among our populations, we plotted the individual relative warp scores for each fish along relative warp 1, relative warp 2, and relative warp 3. Body shape differences along each warp were illustrated by showing the thin plate spline diagrams.

Figure 3: Typical photograph of a brook trout used in the analysis of morphometric variations.

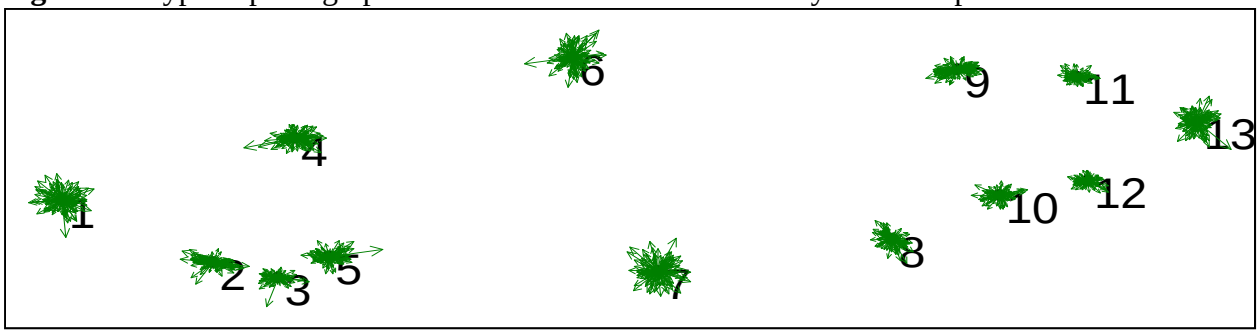


Figure 4: Consensus configuration plot of all trout included in the geometric morphometric analysis.

Preliminary Results

Benthic Macroinvertebrate Communities

Each of the benthic macroinvertebrate sample sites would be listed as impaired by the DEP due to the low IBI score, with the exceptions of HB01 and TM01 both of which are upstream of AMD pollution sources. However, it is interesting to note that MB01, the reference site on Middle Branch upstream from the AMD impairment, also does not have an IBI score that meets DEP requirements. This may very well be indicative of headwater freestone streams that have poor buffering capacity, and as a result the DEP is currently reviewing IBI scores and other metrics with respect to natural, unimpaired conditions in headwater streams. These two sites and MB01 will be used in further research as a benchmark for gauging remediation success for benthic macroinvertebrates.

The remainder of the results that are discussed will be focused on the Middle Branch sample sites because that is the only previously impaired area that is currently experiencing quantifiable recovery (sample site MB02; see Table 3).

Table 2 (a): Biological metrics calculated from benthic macroinvertebrate samples throughout Twomile Run watershed in 2012. *IBI scores are based on DEP protocols. Highlighted IBI scores indicate sites with less than 160 total individuals. A benthic macroinvertebrate sample was not collected at KC02 due to depth and inaccessible habitat. Also, although sampling was attempted at HB02, no macroinvertebrates were found.

Site	**KC 01**	**KC 03**	**TM 01**	**TM 02**	**TM 03**	**TM 04**	**TM 05**	**RH 01**	**RH 02**	**MB 01**	**MB 02**	**HB 01**
Total Abundance	194	18	229	69	37	15	27	30	31	225	124	216
Total Taxa Richness	25	7	26	5	7	7	7	9	9	15	15	25
EPT Taxa Richness (PTV 0-4)	13	1	13	1	2	2	2	3	3	8	8	13
Beck's Index	10	0	29	3	4	7	5	7	8	21	17	31
Hilsenhoff Biotic Index	5.62	5.61	3.09	5.04	4.78	4.73	4.85	3.5	3.55	3.61	3.46	2.56
Shannon Diversity	2.72	1.66	2.64	.66	1.07	1.51	1.12	1.94	1.81	1.61	1.75	2.53
Percent Sensitive Individuals (PTV 0-3)	34.5	11.1	69.9	2.9	24.3	26.7	14.8	30	38.7	47.6	55.6	66.7
IBI Score	60.1	25.3	80.6	19.3	28.8	33.2	27.2	40.8	42.1	55.7	56.7	79

Table 2 (b): Biological metrics calculated from benthic macroinvertebrate collections in 2013. Several sites were not accessible due to construction in the watershed.

Site	**TM 04**	**TM 05**	**MB 02**	**HB 02**
Total Abundance	58	93	235	69
Total Taxa Richness	9	6	21	3
EPT Taxa Richness (PTV 0-4)	0	0	0	0
Beck's Index	7	0	22	0
Hilsenhoff Biotic Index	4.31	6.06	4.80	5.99
Shannon Diversity	1.52	0.46	2.43	0.43
Percent Sensitive Individuals (PTV 0-3)	31.0	0	40.0	0
IBI Score	34.3	13.8	53.0	12.3

In the section of Middle Branch previously impaired by AMD, improvements in the benthic macroinvertebrate communities have been observed. Table 3 shows the biological metrics for this site trending towards the unimpaired control site located upstream of historic AMD pollution. In addition, increases in pollution sensitive taxa have been observed downstream of the treatment system.

Table 3: Biological metrics calculated from benthic macroinvertebrate samples on Middle Branch. *IBI scores are based on DEP protocols. The MB01 sample site was used as a control.

	MB02				**MB01**
	2008	**2010**	**2012**	**2013**	**2012**
Total Abundance	3	46	124	235	225
Total Taxa Richness	3	7	15	21	15
EPT Taxa Richness	1	6	8	9	8
% Sensitive Individuals	33.3	97.8	55.6	40.0	47.6
IBI Score*	30.4	53.9	56.7	53.0	55.7

Fishery Surveys

The sample sites on the mainstem of Kettle Creek each contained species compositions consistent to a warmwater fishery. Within the Twomile Run watershed, brook trout were

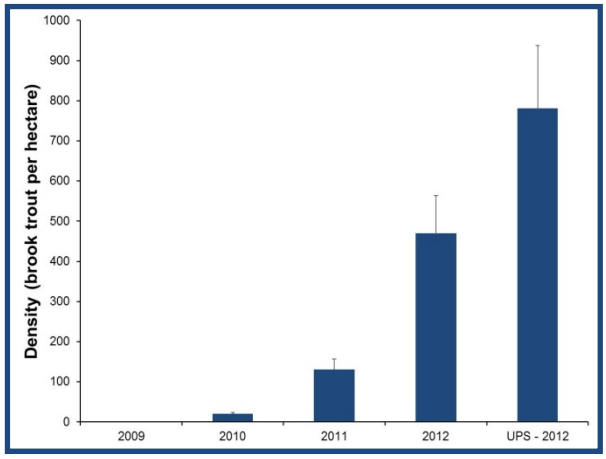
captured at four of the sample sites; TM01, MB01, MB02, and HB01. Sample sites TM01, MB01, and HB01 were known to contain good populations of brook trout since they are upstream of any AMD pollution sources. Brook trout were first documented at MB02 in 2010 (four years after completion of the treatment system) and fishery surveys have been completed here on an annual basis. Table 4 shows brook trout density at each of the sites. It is expected that as each AMD discharge is treated, more downstream habitat will become available for recolonization by brook trout and results similar to those of Middle Branch will be observed.

Figure 5 shows the density and size class distribution of brook trout over time in Middle Branch. No other species were observed in the Twomile Run watershed. Young-of-year brook trout were first observed downstream of the treatment system in 2012 (Figure 5(b)) and brook trout densities have increased each year following treatment. No fishery surveys were completed in 2013 due to construction at the Area 7 reclamation project site, which posed safety concerns and limited access to the sites. Fishery surveys are expected to continue in 2014.

Table 4: Brook trout densities observed in 2012 for sample sites with brook trout. These data will serve as baseline data in monitoring future biological recovery.

Sample Site	Density (trout/ha)
TM01	632
MB01	790
MB02	475
HB01	926

a)



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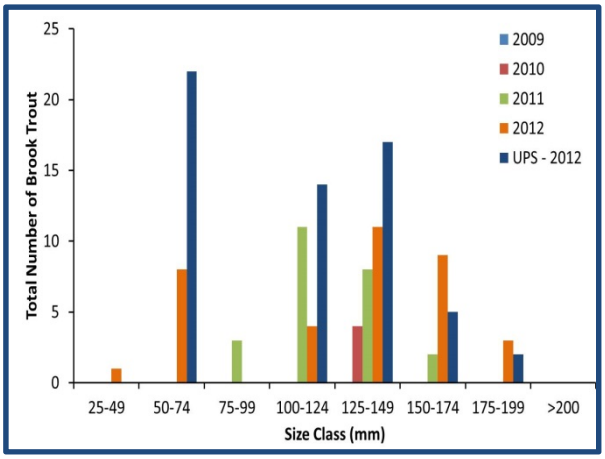

Figure 5: Brook trout (a) density and (b) size class distribution in Middle Branch. The upstream site (UPS; MB01) served as a control.

Brook Trout Morphometrics

Morphological results are primarily based on the results of the relative warp scores that were discussed in the methods section. Figures 6 and 7 depict the relative warp diagrams generated from these analyses. The first three relative warps summarized 48.1% of the total variation in shape among all of the fish. Relative warp 1 comprised 20.7% of the total variation in shape. The thin plate spline diagrams illustrate the upward and downward 'bending' of the fish as relative warp scores progress from negative to positive values (Figure 6). Although care was taken to position each fish before photography, this bending effect was noticeable after referring back to fish that scored large negative and positive values on warp 1.

Relative warp 2 summarized 15.5% of the total variation in shape and summarized shape variation associated with the health of the fish (y-axis, Figure 6). Fish with a low relative warp 2 value tended to be fatter. A high relative warp 2 value correlates with a 'skinnier' fish. Relative warp 3 summarized 11.9% of the total variation in shape and summarizing shape associated with variation in mouth size and shape. Fish with high relative warp 3 values were larger fish with proportionally larger mouths.

Upon investigation of the pattern of relative warp scores from each fish, clusters of trout were quantified based on relative warp score 1 and relative warp score 2. Relative warp 2, describing the overall health of the trout, had three distinct clusters; fish above AMD had lower values, fish below AMD had higher values, and fish from Larrys Creek watershed had the lowest values (Figure 6). Relative warp 3, describing mouth size, indicates that trout with lower values have larger mouths and trout with higher values have smaller mouths. These differences are after correcting for size differences among the fish, thus the axis for relative warp 3 represents fish with proportionately larger mouths at low values and proportionately smaller mouths at higher values (Figures 7 and 8).

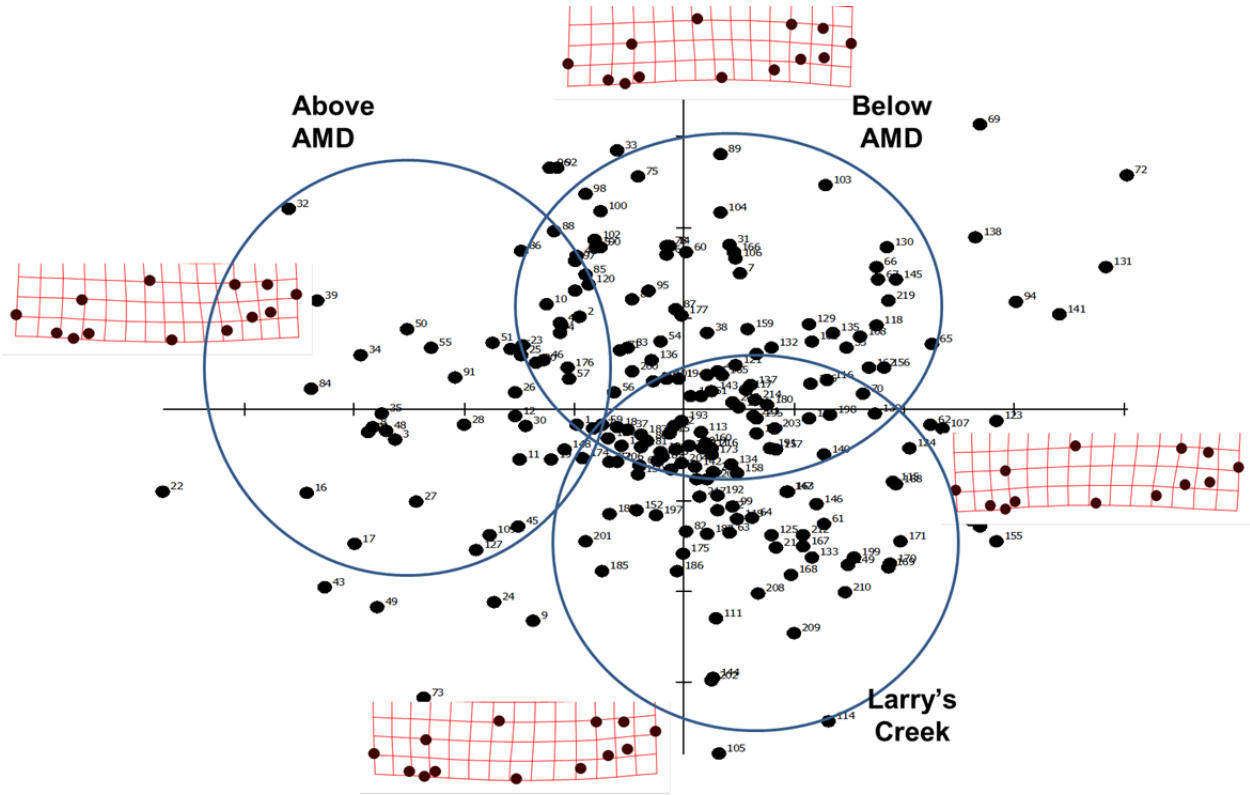

Figure 6: Relative warp diagram showing the differences of fish shape.

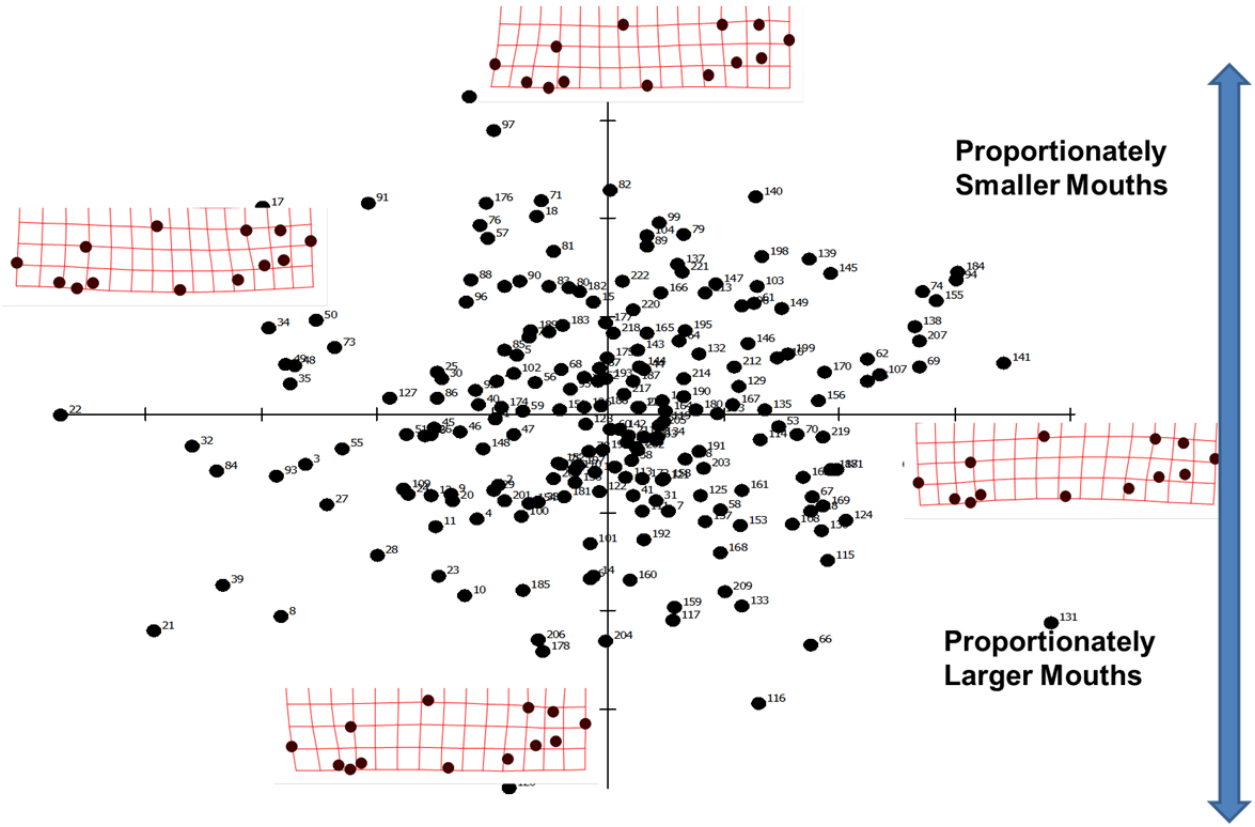

Figure 7: Relative warp diagram showing differences in mouth size and shape.

Figure 8: Photographs of brook trout with proportionally larger mouths (left) and proportionally smaller mouths (right).

After viewing photographs from individual trout across the range of values on the axis for relative warp 1, the bending effect caused by placement on the board was subtle but noticeable. We did not anticipate this variation to be quantified in the relative warps analysis however the result emphasizes the sensitivity to shape differences that are detected by geometric morphometric techniques. The ordering of fish along relative warp 2 differentiates trout along their 'fatness'. Trout with lower values generally have larger bellies and trout with higher values have skinnier bellies. As indicated on Figure 2, the relative warp 2 scores are generally sorted with trout from Larrys Creek having lower values and trout from the locations experiencing mild

AMD impact with higher values. Trout above AMD impact span the range of values on this axis. Therefore, fatness and skinniness could be related to the density and quality of food items in these two locations. The ordering of fish along relative warp 3 differentiates trout along the relative size of their mouth. This suggests that as brook trout grow, their mouths grow proportionally larger as noted by the changes, in relative position of landmarks 1, 2, and 3, between the lower and upper thin plate spline diagrams. This finding may be an example of allometric growth changes that are related to a diet shift towards proportionately larger prey items as trout mature.

Additional Research Projects/Future Research

As each of the remediation projects is completed, it is expected that brook trout will begin to recolonize newly available habitat throughout the Twomile Run watershed. Therefore, the efforts to monitor benthic macroinvertebrate communities and fish populations will continue on an annual basis in the future, provided adequate funding is secured. These data will provide a long-term data set that will provide insight into how these populations recover. In addition, this project will provide the opportunity to evaluate any shortcoming in restoring biological communities in streams previously impaired by AMD or other sources of chemical pollution an offer insight into how these issues may be addressed in the future. In addition to the monitoring and research efforts that have detailed previously, several other projects focused on the biology and ecology of these streams are underway. These projects are briefly described below.

Brook Trout Movement

It is expected that as water quality continues to improve throughout the watershed that brook trout will begin to recolonize previously unoccupied sections of the watershed (similar to results in Middle Branch below the treatment system). Currently there are at least three distinct brook trout populations in the watershed that have been isolated from one another due to AMD pollution. It will be important to gain an understanding of which populations are contributing to the recolonization of new areas of Twomile Run. To this end, during the 2012 fishery surveys, all fish captured that were greater than 100 mm in total length were tagged with a visible Flow T-Bar Anchor tag. Each tag was uniquely numbered and color coded by the stream reach the fish was originally captured. Fishery surveys were not completed in 2013 therefore there are no results to report on from this study to date. Tagged fish data will be included in the 2014 fishery survey as well as future surveys.

Brook Trout Genetics

In addition to tagging fish to monitor movement, another popular method to determine if previously isolated populations are interbreeding following the removal of a barrier is to study the genetics of the populations. Small tissue samples were obtained from the upper caudal fin of each brook trout captured during the 2012 electrofishing surveys. These samples will be used to analyze the genetics of the brook trout populations in Huling Branch, Middle Branch, and upper Twomile Run. Genetic samples are currently being analyzed by Grove City College, following USGS protocols. Samples will be analyzed for both mitochondrial and microsatellite DNA.

Genetic samples will continue to be collected from brook trout in subsequent surveys. Over time, it is expected that the genetics of these once isolated populations will begin to resemble one another following the removal of barriers. Over time, the genetic data will determine if brook trout are interbreeding and genetic diversity of the population is increasing, an important metric for population stability and conservation.

Brook Trout Morphometrics

The first year of this study has provided some interesting results with respect to differences found in trout inhabiting waters previously impaired by AMD and those inhabiting unimpaired waters. However, these results remain inconclusive and further research is needed to understand the implications of these results. Therefore, we plan to expand the sampling efforts to additional brook trout populations from interconnected systems and populations from areas with moderate AMD impairment to better assess whether our initial patterns in 'fatness' and skinniness' are consistent. Further investigation of the drifting invertebrates within these areas could corroborate that trout living in moderate AMD impairment experience lower available energetic inputs.

On a broader ecological scale, our finding that smaller fish have smaller than average mouth size and larger fish have larger than average mouth size may be an example of allometric growth pattern that has not been reported in the scientific literature for brook trout. Again, expanding the scope of this project will allow us to further investigate the possibility of allometric growth. In addition, mouth shape and size is often a sex-linked characteristic in brook trout. Therefore, we are planning to investigate whether the sex of the trout is related to this pattern and also if sex ratios for brook trout vary between previously impaired AMD reaches and unimpaired reaches of the stream. Finally, the genetic data obtained will also be incorporated into the morphometric data set to determine if the morphological differences that have been observed are due to genetics or some other environmental factor.

In summary, Twomile Run provides one of the first opportunities to intensively study the biological recovery of stream previously impaired by AMD at various ecology levels (from individual to community levels of organization). Although, biological recovery requires a greater length of time than that of water quality changes (see Figure 9 for an overview of the expected time frame for the initial stages of biological recovery), the data from these studies will provide valuable direction to future restoration and conservation efforts in areas suffering from AMD and other types of pollution. In addition, these studies also document the importance of funding to be provided for long-term monitoring of biological communities, particularly for projects with the ultimate objective of restoring a sustainable fishery.

SUMMARY

Overall, the biological recovery of the Twomile Run watershed remains in its infancy, as several AMD remediation projects have just been completed or remain to be completed. The first significant signs of recovery have been documented in Middle Branch. Since the completion of the Middle Branch treatment system, benthic macroinvertebrates have begun to recolonize the area that was previously impaired by AMD. Increases have been noted in the numbers of

pollution sensitive taxa such as mayflies, stoneflies, and caddisflies. In addition, brook trout populations have also begun to recolonize the area downstream of the treatment system. The first brook trout were observed in this reach three years after the completion of the treatment system. The most recent fishery surveys (2012) noted several size classes of brook trout, including young-of-the-year, which indicates that successful reproduction is occurring. It is expected that the recovery of this system will continue as water quality continues to improve throughout the watershed. The monitoring efforts that were initiated in the last several years will provide information valuable for the successful implementation of projects with a focus on restoring biological communities in watersheds impaired by AMD or other pollution sources.

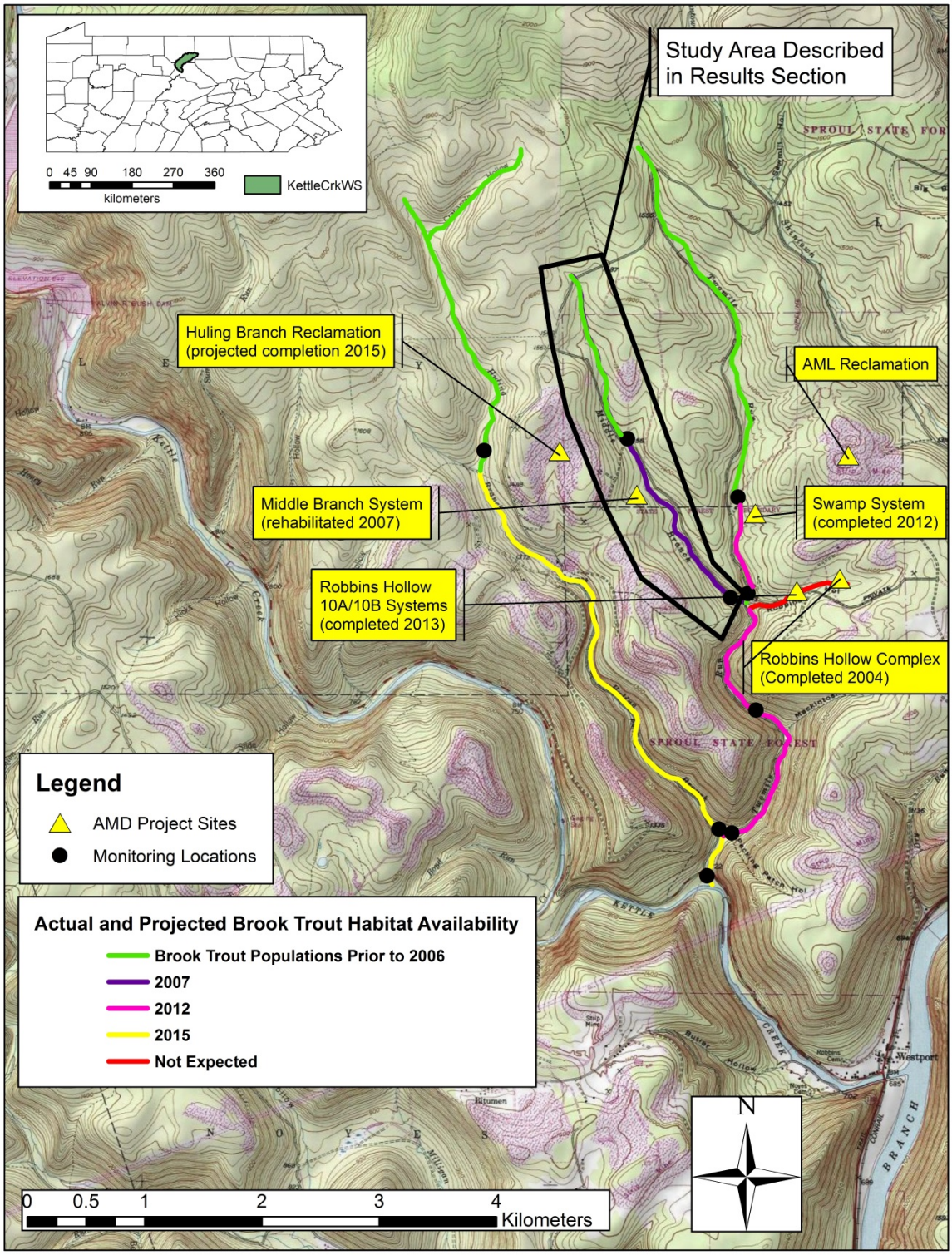

Figure 9: Map depicting restoration efforts in the Twomile Run watershed. AMD treatment systems and reclamation projects are shown in yellow. Streams are color coded based on documented brook trout populations and projected available habitat as projects are completed.

APPENDIX A: Description of biological metrics that were used in this project.

Total Abundance

The total abundance is the total number of organisms collected in a sample or sub-sample.

Dominant Taxa Abundance

This metric is the total number of individual organisms collected in a sample or sub-subsample that belong to the taxa containing the greatest numbers of individuals.

Taxa Richness

This is a count of the total number of taxa in a sample or sub-sample. This metric is expected to decrease with increasing anthropogenic stress to a stream ecosystem, reflecting loss of taxa and increasing dominance of a few pollution-tolerant taxa.

% EPT Taxa

This metric is the percentage of the sample that is comprised of the number of taxa belonging to the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT). Common names for these orders are mayflies, stoneflies, and caddisflies, respectively. The aquatic life stages of these three insect orders are generally considered sensitive to, or intolerant of, pollution (Lenat and Penrose 1996). This metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting the loss of taxa from these largely pollution-sensitive orders.

Shannon Diversity Index

The Shannon Diversity Index is a community composition metric that takes into account both taxonomic richness and evenness of individuals across taxa of a sample or sub-sample. In general, this metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting loss of pollution-sensitive taxa and increasing dominance of a few pollution-tolerant taxa.

Hilsenhoff Biotic Index

This community composition and tolerance metric is calculated as an average of the number of individuals in a sample or sub-sample, weighted by pollution tolerance values. The Hilsenhoff Biotic Index was developed by William Hilsenhoff (Hilsenhoff 1977, 1987; Klemm et al. 1990) and generally increases with increasing ecosystem stress, reflecting dominance of pollution-tolerant organisms. Pollution tolerance values used to calculate this metric are largely based on organic nutrient pollution. Therefore, care should be given when interpreting this metric for stream ecosystems that are largely impacted by acidic pollution from AMD or acid deposition.

Beck's Biotic Index

This metric combines taxonomic richness and pollution tolerance. It is a weighted count of taxa with PTVs of 0, 1, or 2. It is based on the work of William H. Beck in 1955. The metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting the loss of pollution-sensitive taxa.

Percent (%) Sensitive Individuals

This community composition and tolerance metric is the percentage of individuals with PTVs of 0 to 3 in a sample or sub-sample and is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting the loss of pollution-sensitive organisms

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