DRAFT WADEABLE FREESTONE ACIDIFICATION ASSESSMENT METHOD

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INTRODUCTION

This assessment method is designed to make Aquatic Life Use (ALU) and Special Protection (SP) assessment decisions using benthic macroinvertebrate and/or fish communities in Pennsylvania's headwater freestone, riffle-run streams. Full technical documentation of this assessment method can be found in the *Wadeable Freestone Acidification Assessment Method Technical Report* (Shank 2023). This method is meant to supplement the *Wadeable Freestone Riffle-Run Macroinvertebrate Assessment Method* (Shull 2023) and the *Stream Fish Assemblage Assessment Method* (Wertz 2021) but is independently applicable when making assessment decisions. These existing fish and macroinvertebrate tools are well calibrated to make assessments in these settings; however, each method contains considerations when acidification impacts are suspected.

Surface water acidification is a process characterized by increasing concentrations of hydrogen ions (H^+), which lowers the pH of waterbodies. Metals and their compounds can ionize in response to acidification, which can produce ions (e.g., Al^{3+}) in concentrations high enough to be toxic to aquatic life. As a result, increasing acidification is indicative of environmental degradation. Forested headwater streams underlain by base-cation poor geology are prone to surface water acidification. Centuries of fossil fuel combustion has led to atmospheric deposition (AD) of acidic anions such as sulfate (SO_4) and nitrate (NO_3), which has depleted soils of base cations in these settings. Although AD of acidic anions has greatly decreased due to the Clean Air Act, headwater streams continue to exhibit depressed pH and alkalinity and elevated aluminum (Al) concentrations, which can greatly affect biological communities (Driscoll et al. 2001). Acid mine drainage (AMD) is a similar, prevalent stressor in forested, headwater settings. The acidity from both AD and AMD is derived mainly from sulfuric acid (H_2SO_4), which leads to similar water quality, especially when streams are slightly affected by AMD (Herlihy et al. 1990, Herlihy et al. 1991). This acidification assessment method employs metrics that are calibrated to recognize the biological effects of surface water acidification. Macroinvertebrate and fish communities integrate water quality stress at acidified sites, which provides advantages over instantaneous measurements of discrete water quality (Barbour et al. 1999). However, water chemistry data collections are imperative to determine whether the source of acidification is AD or AMD, as each source contains predictably different concentrations of key parameters (Merovich et al. 2007).

The *Wadeable Freestone Riffle-Run Macroinvertebrate Assessment Method* (Shull 2023) includes a series of qualifier questions that must be considered when making assessments even when index of biotic integrity (IBI) scores do not exceed impairment thresholds. The macroinvertebrate assessment method flowchart has been modified to synchronize with this new acidification assessment method. Specifically, the fourth qualifier question formerly asked if samples show signatures of acidification (Figure 1a). The acidification method will replace this qualifier question and provide specific guidance on assessment and source and cause decisions due to acidification (Figure 1b). Future wadeable freestone assessments using macroinvertebrate communities should consult this acidification assessment method as a final step in an assessment.

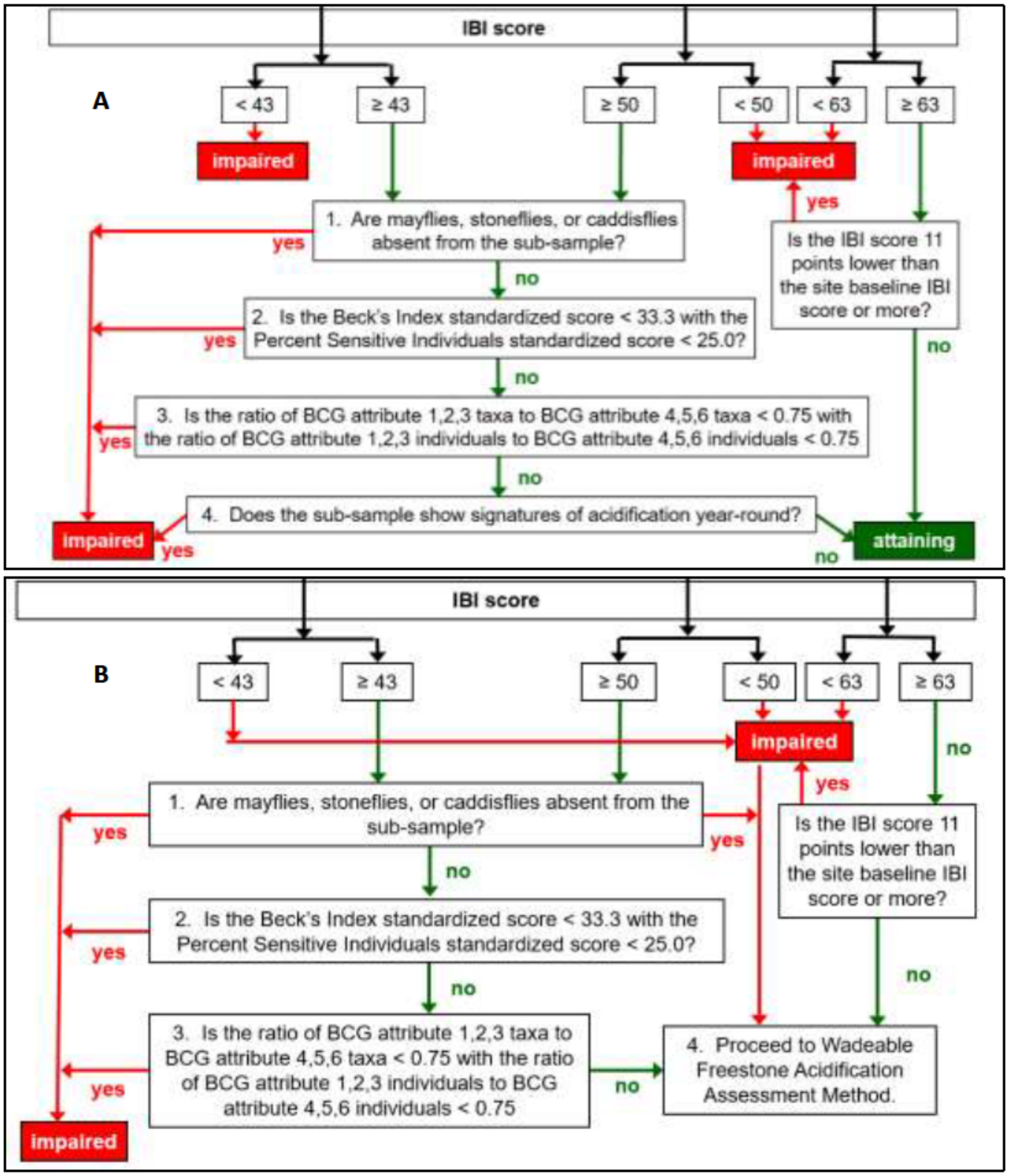

Figure 1. Changes to the *Wadeable Freestone Riffle-Run Macroinvertebrate Assessment Method* (Shull 2023) flowchart. These changes acknowledge that the Wadeable Freestone Acidification Assessment Method should be considered before any assessment decisions can be made using the Freestone IBI. It should also be used in most cases when the Freestone IBI is used to make an impairment decision. A) The original assessment flowchart where acidification was generally considered through macroinvertebrate community characteristics and Special Protection streams were not specifically included in acidification considerations. B) Adjustment to the assessment flowchart that provides additional acidification considerations using the Wadeable Freestone Acidification Assessment Method.

The *Stream Fish Assemblage Assessment Method* (Wertz 2021) contains guidance on community signatures of acidification impacts that can result in impairment even if thermal fish index (TFI) scores do not exceed impairment thresholds. This method should be pursued when <50 individuals or salmonid-only communities are present. Water chemistry data is necessary to accurately determine sources and causes of impairment in all situations.

ASSESSMENT REQUIREMENTS

Prior to data collection, sample design should follow guidance in *Stream and River Assessment Survey Design* (Shull and Arnold 2023b), where sampling locations are placed to represent waterbody condition on small tributaries at an appropriate resolution to reflect influences of land cover, geology, and abandoned mine lands (AML).

To use this method for assessment and source/cause decision purposes, data collection must follow applicable protocols established in the Monitoring Book (Lookenbill and Arnold 2023). A minimum of one discrete water chemistry sample must be collected during or near the time of biological sampling following the *Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023a). Fish or benthic macroinvertebrate community data is required to use this method. Macroinvertebrates should be collected using the *Wadeable Riffle Run Stream Macroinvertebrate Data Collection Protocol* (Shull 2017) and subsampled using the *Macroinvertebrate Laboratory Subsampling and Identification Protocol* (Brickner 2020). Fish should be sampled using the *Fish Data Collection Protocol* (Wertz and Arnold 2023).

The *Wadeable Freestone Riffle-Run Macroinvertebrate Assessment Method* (Shull 2023) and/or the *Stream Fish Assemblage Assessment Method* (Wertz 2021) and their applicable impairment thresholds must be implemented prior to using this acidification assessment method. The *Discrete Physicochemical Assessment Method* (Biggs and Whiteash 2021) should be implemented. A simplified flowchart of the sample design, data collection, and assessment process is shown in Figure 2.

This acidification method should only be applied in applicable settings, defined as headwater streams (1^{st}-4^{th} order) in drainages of <25 mi^2 with high gradient habitat. The influence of bogs and/or wetlands should be limited, as those habitats contribute organic acidity to receiving streams that may be conflated with acidity of anthropogenic origins. These streams are often tannic and as a result of depressed pH, biological communities can resemble those of AD or AMD impacted streams without the presence of base-poor geology or mining influence. The development dataset was limited to watersheds with <25% of land area comprised of palustrine wetlands and streams with <10 mg/L total organic carbon (TOC). These thresholds of wetland coverage and TOC concentrations represent upper limits for inclusion of samples and sites into this assessment method.

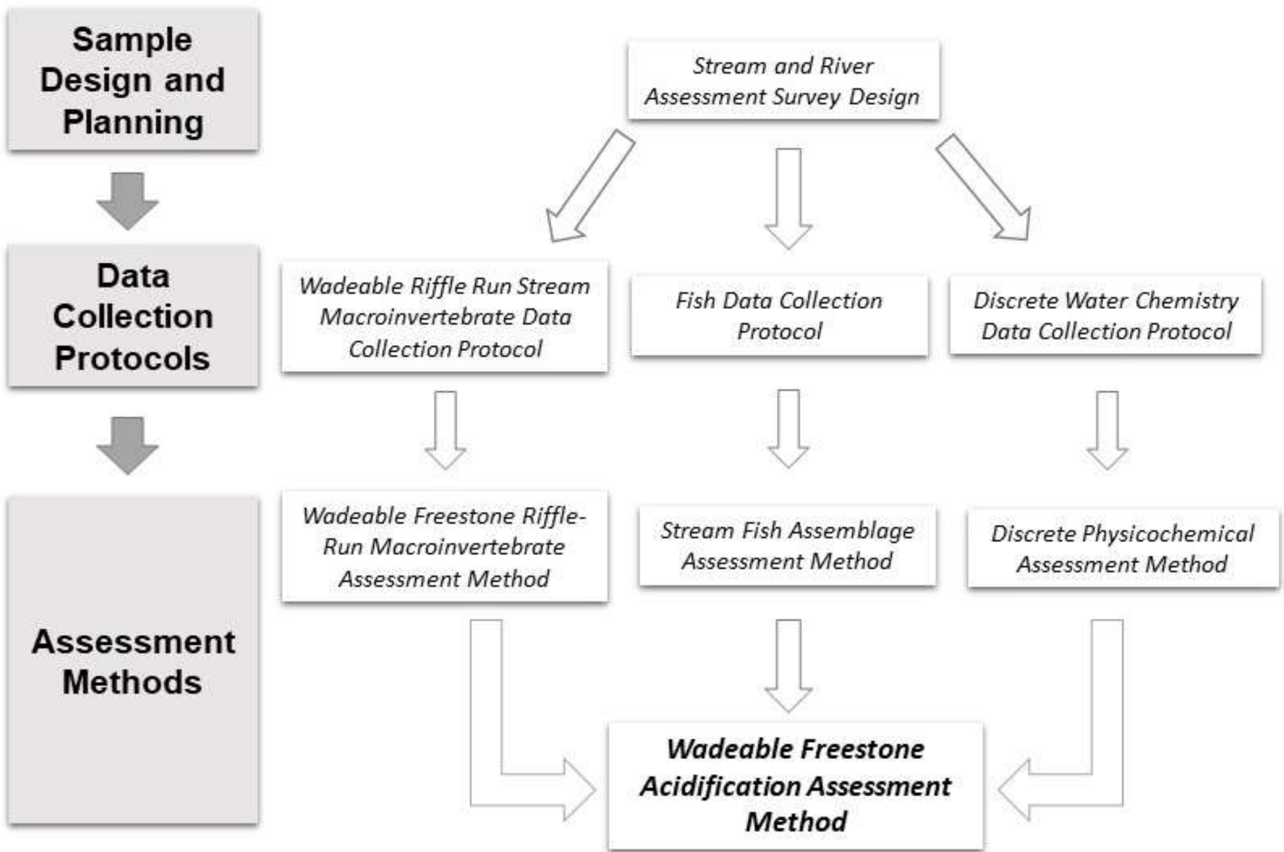

Figure 2. Sample design, data collection, and assessment process required when using the wadeable freestone acidification assessment method.

THE METRICS

Fish and macroinvertebrate metrics can be used to successfully employ this method. Each metric adequately discriminates between streams affected by acidification and non-acidified streams. These metrics add information to existing assessment methods and promote accurate source and cause decisions. Either fish or macroinvertebrate community data should be collected; only one community is needed, but both may increase clarity of site conditions.

Macroinvertebrate Metrics

Acid Tolerance Index Calculation

Taxon-specific Acid Tolerance Values (ATVs) ranging from 0-10 (low-high acid tolerance) were derived for 112 macroinvertebrates (Shank 2023). The Acid Tolerance Index (ATI) is the sum product of the abundance of each taxon and its ATV, which represents the sample's community acid tolerance on a 0 – 100 scale, with low values showing low community acid tolerance and high values indicating large proportions of acid tolerant taxa.

Example calculations for the ATI are provided below for a macroinvertebrate community sample collected on March 18, 2008 from Panther Run in Centre County (Table 1). ATI calculations are appropriate when >90% of individuals in the subsample have an ATV.

Table 1. Benthic macroinvertebrate subsample from Panther Run, Centre County collected on March 18, 2008 (20080318-1017-rryder).

TSN	Order	Family	Assessment ID	Number of Individuals	ATV
114244	Coleoptera	Elmidae	*Oulimnius*	34	2
114229	Coleoptera	Elmidae	*Promoresia*	1	3
127917	Diptera	Chironomidae	Chironomidae	15	4
136305	Diptera	Empididae	*Chelifera*	1	2
126703	Diptera	Simuliidae	*Prosimulium*	5	5
119656	Diptera	Tipulidae	*Antocha*	1	2
100800	Ephemeroptera	Baetidae	*Baetis*	4	1
568550	Ephemeroptera	Baetidae	*Diphetor*	1	2
101365	Ephemeroptera	Ephemerellidae	*Drunella*	5	1
101233	Ephemeroptera	Ephemerellidae	*Ephemerella*	11	2
101324	Ephemeroptera	Ephemerellidae	*Eurylophella*	1	5
100557	Ephemeroptera	Heptageniidae	*Cinygmula*	4	0
100626	Ephemeroptera	Heptageniidae	*Epeorus*	7	1
697957	Ephemeroptera	Heptageniidae	*Maccaffertium*	5	3
101187	Ephemeroptera	Leptophlebiidae	*Paraleptophlebia*	6	1
101766	Odonata	Gomphidae	*Lanthus*	2	3
102844	Plecoptera	Leuctridae	*Leuctra*	45	9
102540	Plecoptera	Nemouridae	*Amphinemura*	16	9
102917	Plecoptera	Perlidae	*Acroneuria*	1	2
102471	Plecoptera	Pteronarcyidae	*Pteronarcys*	2	1
115399	Trichoptera	Hydropsychidae	*Diplectrona*	3	5
116794	Trichoptera	Lepidostomatidae	*Lepidostoma*	1	4
115319	Trichoptera	Philopotamidae	*Dolophilodes*	2	2
115097	Trichoptera	Rhyacophilidae	*Rhyacophila*	8	5
116046	Trichoptera	Uenoidae	*Neophylax*	1	4
568832			Clitellata	25	5

Sample ATI Score = $\frac{\Sigma n_i \; x \; ATV_i}{N}$ * 10

Where, *n* is the number of individuals of taxa *i*, *ATV* is the acid tolerance value (ATV) of taxa *i*, and *N* is the total number of individuals in the sample with an ATV.

In this subsample,
there are 4 individuals with ATV = 0,
there are 24 individuals with ATV = 1,
there are 51 individuals with ATV = 2,
there are 8 individuals with ATV = 3,
there are 17 individuals with ATV = 4,
there are 42 individuals with ATV = 5,
there are 0 individuals with ATV = 6, 7, or 8,
there are 61 individuals with ATV = 9, and
there are 0 individuals with ATV = 10.

There is a total of 207 individuals in this sub-sample, so
Acid Tolerance Index = ([(0 * 4) + (1 * 24) + (2 * 51) + (3 * 8) + (4 * 17) + (5 * 42) + (6 * 0) + (7 * 0) + (8 * 0) + (9 * 61) + (10 * 0)] / 207) * 10
Acid Tolerance Index = **47.2**

Fish Metrics

Total Individuals and ***FishGenera***$_{AcidIntol}$ Calculations

The fish metrics used for acidification assessment are the total number of individuals in the sample and the sum of *Cottus, Etheostoma, Clinostomus,* and *Oncorhynchus* relative abundance (***FishGenera***$_{AcidIntol}$). Example calculations for these metrics are provided below for a fish community sample collected on July 7, 2022 from Nickel Run in Tioga County (Table 2). If sampling reveals no fish present, these metrics are both equal to zero.

Table 2. Fish community sample from Nickel Run, Tioga County collected on July 7, 2022 (20220707-0832-dushull).

TSN	Family	Genus	Scientific Name	Common Name	Number of individuals
161997	Salmonidae	*Salmo*	*Salmo trutta*	brown trout	7
162003	Salmonidae	*Salvelinus*	*Salvelinus fontinalis*	brook trout	47
167232	Cottidae	*Cottus*	*Cottus cognatus*	slimy sculpin	48
163382	Cyprinidae	*Rhinichthys*	*Rhinichthys atratulus*	eastern blacknose dace	9
163384	Cyprinidae	*Rhinichthys*	*Rhinichthys cataractae*	longnose dace	13

Total Individuals = Σ Number of individuals
In this sample, 7 + 47 + 48 + 9 + 13 = 124

$$\textbf{\textit{FishGenera}}_{AcidIntol} = \frac{\Sigma\, Cottus,\ \ Etheostoma,\ \ Clinostomus,\ \text{ and } Oncorhynchus \text{ individuals}}{\text{Total Individuals}} * 100$$

In this sample, 48 *Cottus* individuals / 124 total individuals * 100 = 38.7%

Water Chemistry Metrics

Water chemistry parameters displayed adequate discrimination to separate AD and AMD sources of surface water acidification. These parameters must be collected and evaluated against the established concentrations in Table 3 to determine the appropriate source using a weight of evidence approach. Collection of chemistry concurrently during biological sampling is recommended. If sampling logistics do not allow simultaneous collection of biology and chemistry, sampling chemistry as near to the time of biological sampling is acceptable. More than one chemistry sample is also recommended to appropriately capture the range of conditions experienced at a site, whenever possible (Shull and Arnold 2023b).

Water Chemistry Evaluation

The 10th and 90th percentiles of water chemistry parameters were calculated for a population of sites where the sole stressor was AD. These parameter concentrations can discriminate between AD and AMD sources of acidification. Macroinvertebrate and fish communities and metrics often look similar at sites affected by AD and AMD. As such, the water chemistry parameters with sufficient discrimination can be used along with field and desktop reconnaissance to determine the appropriate source of impairments, consistent with the process defined in the *General Source and Cause Assessment Method* (Shull and Shank 2023). The parameters with sufficient discrimination to separate AD and AMD sources include dissolved (d) and total (t) calcium, t dissolved solids, t hardness, d and t iron, d and t magnesium, d and t manganese, d and t potassium, t sodium, SpC, t sulfate, and t and d zinc (Table 3).

Table 3. The 10th – 90th percentile range of parameter concentrations at sites where the sole stressor is atmospheric deposition (AD Range). Parameters that effectively discriminate between acid mine drainage (AMD Indicator) and non-impacted sites (Non-Impacted Indicator) are shown, including concentration thresholds and directionality.

Parameter (units)	AD Range	AMD Indicator	Non-Impacted Indicator
Acidity Total (mg/L)	3.0 - 10.0		<3.0
Alkalinity (Lab) (mg/L)	<3.0		>3.0
Aluminum Dissolved (µg/L)	31 - 261		
Aluminum Total (µg/L)	62 - 366		
Calcium Dissolved (mg/L)	<2.0	>2.0	>2.0
Calcium Total (mg/L)	<2.0	>2.0	>2.0
Dissolved Solids Total (mg/L)	20 - 30	>30.0	>30.0
Hardness Total (mg/L)	3.0 - 7.0	>7.0	>7.0
Iron Dissolved (µg/L)	20 - 87	>87.0	
Iron Total (µg/L)	21 - 156	>156.0	
Magnesium Dissolved (mg/L)	<1.0	>1.0	>1.0
Magnesium Total (mg/L)	<1.0	>1.0	>1.0
Manganese Dissolved (µg/L)	17.0 - 180.0	>180.0	<17.0
Manganese Total (µg/L)	11.0 - 100.0	>100.0	
pH (Field) (pH units)	4.8 - 5.9		>5.9
Potassium Dissolved (mg/L)	<1.0	>1.0	
Potassium Total (mg/L)	<1.0	>1.0	
Sodium Total (mg/L)	<1.0	>1.0	
Specific Conductance (Field) (µs/cm)	16 - 30.0	>30.0	>30.0
Sulfate Total (mg/L)	3.0 - 7.0	>7.0	
Zinc Dissolved (µg/L)	10.0 - 23.0	>23.0	
Zinc Total (µg/L)	10.0 - 19.0	>19.0	

ASSESSMENT THRESHOLDS

After evaluating physical, chemical, and biological data against all applicable assessment methods, including the *Wadeable Freestone Riffle-Run Macroinvertebrate Assessment Method* (Shull 2023) and/or the *Stream Fish Assemblage Assessment Method* (Wertz 2021), the wadeable freestone acidification assessment should occur. A simplified flowchart of this decision-making process is outlined in Figure 3. Although this simplified decision matrix should guide most assessment decisions for benthic macroinvertebrate or fish samples, situations exist where this simplified assessment schematic will not apply exactly as outlined – some such situations are discussed in the following text.

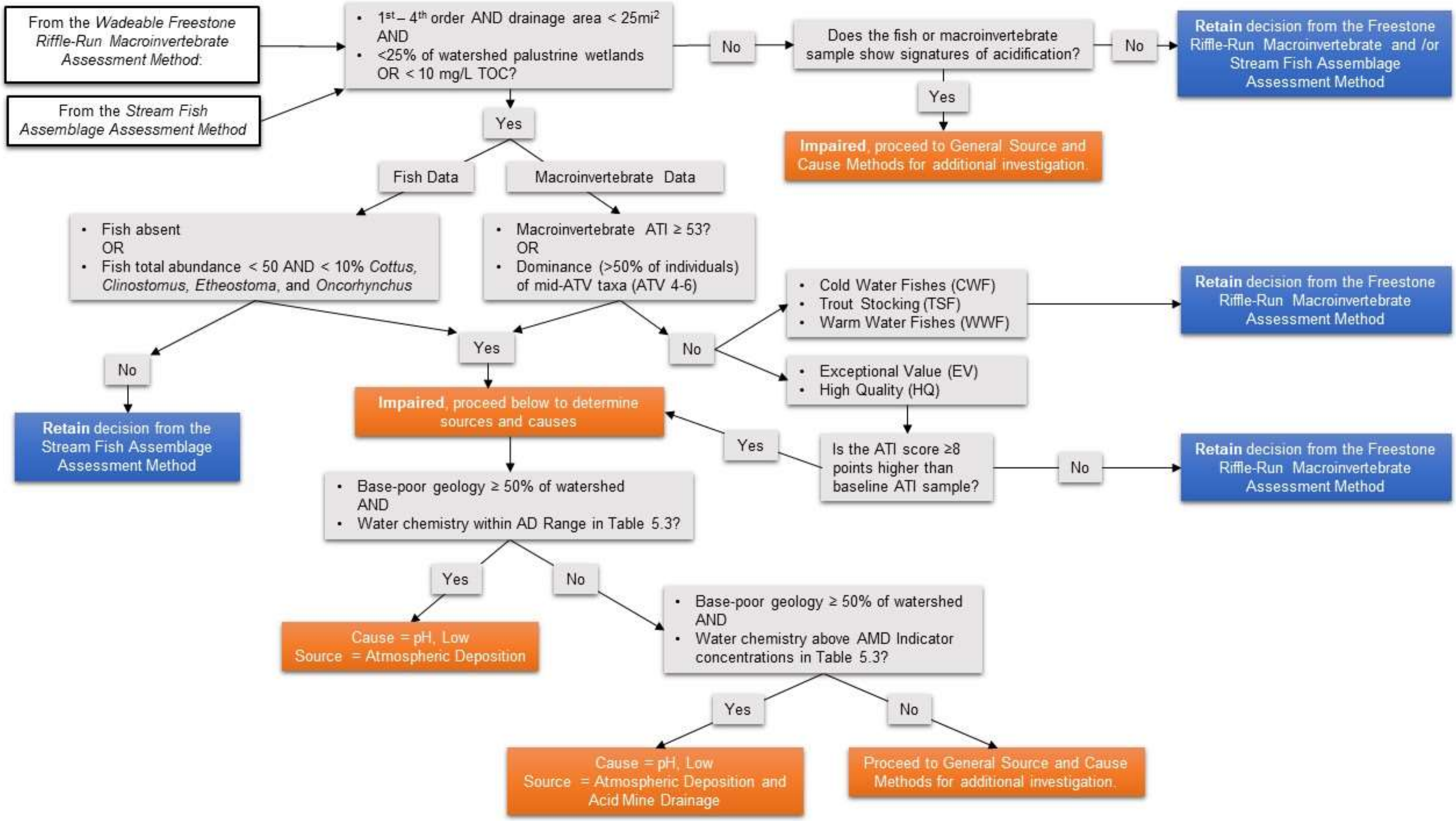

Figure 3. Assessment flowchart for the wadeable freestone acidification assessment method.

Applicable Setting Thresholds

Applicable settings for the surface water acidification method are:

- headwater streams (1st-4th order) in drainages of <25 mi^2 with high gradient habitat; and
- watersheds with <25% of land area comprised of palustrine wetlands or <10 mg/L TOC concentrations.

These small headwater settings are most prone to acidification impacts and is consistent with the dataset on which this method was built. All sites that are >4th order and ≥25mi^2, or have ≥25% of wetland area in their watershed, or ≥10 mg/L should rely on narrative considerations describing biological indicators of acidification in *Wadeable Freestone Riffle-Run Macroinvertebrate Assessment Method* (Shull 2023) and/or the *Stream Fish Assemblage Assessment Method* (Wertz 2021).

Special Considerations

<u>Dominant Taxa</u>

When water chemistry is strongly influenced by AMD with elevated acidity, metals, SO_4, and low pH, increased dominance (>50% of individuals) of a moderately tolerant taxon (ATV 4-6; e.g., Chironomidae) can occur. This can result in macroinvertebrate communities with low richness (<15 taxa), supporting ATI scores <53 and often occurs in combination with impaired freestone IBI scores. In these instances, water chemistry should be checked against AMD indicators in Table 3 and the *General Source and Cause Method* (Shull and Shank 2023) should be completed to determine appropriate sources and causes of impairment.

<u>Barriers</u>

Due to the flight capabilities of adult stages of macroinvertebrates, instream barriers are likely not prohibiting colonization. However, situations do occur where water quality is supporting, but fish communities are salmonid-only or absent due to barriers to migration. A desktop and field reconnaissance should be referenced to evaluate barriers. Geospatial data from the *Comprehensive Aquatic Barrier Inventory and Prioritization Tool* (Southeast Aquatic Resources Partnership 2024) and/or the *Stream Crossing Explorer* (North Atlantic Aquatic Connectivity Collaborative 2023) should be consulted for a regional source of barrier locations and severity. When total individuals <50 and ***FishGenera**$_{AcidIntol}$* <10% even though water quality appears supporting (referencing Non-Impacted Indicators in Table 3) and base-poor geology and AML/AMD are absent, but evidence of barriers is found, the acidification method should not be used to make assessment and source and cause decisions. Rather, the *Stream Fish Assemblage Assessment Method* (Wertz 2021) should be used for assessment decisions and the *General Source and Cause Method* (Shull and Shank 2023) should be completed to determine appropriate sources and causes.

Aquatic Life Use Thresholds

When sampling is conducted outside of an applicable setting, the wadeable freestone acidification assessment method should not be used. Instead, when macroinvertebrates and/or fish communities show signs of acidification (described below), and the sample is representative of site conditions, the waterbody is impaired and the *General Source and Cause Assessment Method* (Shull and Shank

2023) should be consulted to determine the appropriate sources and causes of impairment. Habitat conditions (focusing on barriers to migration) should be investigated thoroughly.
Primary signatures of acidification in macroinvertebrate communities include low mayfly abundance and low mayfly diversity (i.e., scarce mayfly individuals and few mayfly taxa), especially when combined with high abundance of *Amphinemura* and/or *Leuctra* stoneflies, occasionally combined with high abundance of Simuliidae and/or Chironomidae individuals. A sub-sample with < 3 mayfly taxa, < 5% mayfly individuals, and > 25% *Leuctra* and/or *Amphinemura* stoneflies indicates likely acidification impacts (Shull 2023).

Fish community data can be used to assess acidification impacts independently, or jointly with macroinvertebrate data. A fish sample represented by only one species (or family for salmonids) is generally considered abnormal even in naturally depauperate headwater streams. This is especially true when sampling in larger streams ≥25 mi^2. When trout are the only species (or family) present in a sample, this is often an indicator of acidified conditions because many other species found in cold water environments have a lower tolerance to acidity (and associated effects) than trout (Johnson et al. 1987, Baker et al. 1996). This salmonid-dominated cold-water assemblage has been an indicator of acidified conditions not only in the Northeastern US (Baker et al. 1996) but across multiple continents (Schofield 1976, Wertz 2021).

In applicable settings, the ALU assessment will consider whether the macroinvertebrate and/or fish metrics exceed the thresholds established in this method. ALU impairment will result from:

- **$FishGenera_{AcidIntol}$** < 10% and total individuals < 50; or
- Fish are absent; or
- ATI scores are ≥53; or
- ATI scores are <53 and community is dominated (>50% of individuals) by a moderately tolerant taxon.

These metrics are calibrated to acidification stress. It is important to note that even if pH is >6 during the time of sampling, exceedance of biological metrics is indicative of low pH, acidified conditions. Instantaneous pH can be elevated during periods of stable flow and/or summer/fall at AD sites but becomes depressed for extended periods during snowmelt and intense precipitation events. The biological metrics are calibrated to respond to pH over the lifespan of macroinvertebrates and fishes, which provides a more comprehensive picture of conditions at the site.

If fish metrics do not exceed thresholds, the decisions from the *Stream Fish Assemblage Assessment Method* (Wertz 2021) should be retained. If macroinvertebrate metrics do not exceed thresholds and the stream is not Special Protection, the decisions from the *Wadeable Freestone Riffle-Run Macroinvertebrate Assessment Method* (Shull 2023) should be retained.

Special Protection Thresholds
In streams with Special Protection uses of High Quality Waters (HQ) or Exceptional Value Waters (EV), DEP considers baseline surveys in wadeable freestone acidification assessments. DEP will protect special protection streams based on a baseline ATI score determined by previous surveys.

Subsequent samples from HQ and EV streams will be compared to the baseline ATI score for a given site using the ATI temporal precision estimates (CI_{90} PE_T; Shank 2023). An ≥8 point increase in ATI scores at the same site represents a substantial change toward acidification and would be considered impaired. If scores decrease by ≥8 points, that represents an improvement and may indicate that a site is recovering from acidification. Furthermore, any sample from an HQ or EV stream that scores ≥53 on the ATI will be considered impaired without compelling reasons otherwise.

If the ATI score is <53 and there is a <8 point increase in ATI scores, the decisions from the *Wadeable Freestone Riffle-Run Macroinvertebrate Assessment Method* (Shull 2023) should be retained.

Source and Cause Decisions

When biological conditions indicate impairment using the wadeable freestone acidification method, the cause of impairment is pH, Low.

Water chemistry and watershed characteristics are used to determine the source of impairment. If ≥50% of the watershed is underlain by base-poor geology and all water chemistry parameters are consistent with the AD Range of Table 3, the likely sole source of impairment is AD.

If base-poor geology is ≥50% of the watershed and water chemistry parameter concentrations are greater than AMD Indicator concentrations in Table 3, it’s likely that additional stressors are responsible for or exacerbating acidified conditions at the site. For example, if Fe, Mn, SO_4, and SpC exceed AMD Indicator concentrations, it is likely that AMD is responsible for a portion of the site acidification. The directionality of chemistry exceedances should be scrutinized, and field and desktop reconnaissance should search for AMD point discharges or abandoned mine lands (AML) in the watershed, as well as other potential sources of acidification. If the watershed is dominated with base-poor geology, water chemistry is consistent with AMD Indicators in Table 3, and AML/AMD is present in the watershed, AD and AMD are the sources of impairment.

If base-poor geology is <50% of watershed area or water chemistry concentrations are not consistent with AMD indicators in Table 3, the *General Source and Cause Assessment Method* (Shull and Shank 2023) should be consulted to determine the appropriate sources and causes of impairment. This scenario is unlikely, as the predominant sources of acidification in small, forested, headwater streams are AD and AMD (Herlihy et al. 1991, Walsh et al. 2007). The potential for biological communities resembling AD or AMD sites exists when wetlands/bogs to contribute organic acidity to streams and/or barriers prevent fish from migrating into suitable habitat, which were detailed in the Special Considerations section.

Only one water chemistry sample is required to implement the wadeable freestone acidification assessment method. However, increased sampling will bring additional clarity to the sources and causes (or lack thereof) of acidification, especially if the additional samples occur during critical periods (e.g., winter/spring snowmelt or intense seasonal precipitation events). If a single chemistry sample does not yield sufficient clarity and biological metrics are near thresholds, additional sampling should be considered.

ASSESSMENT EXAMPLE – MACROINVERTEBRATES

Applicable Setting Determination

Four watersheds in northern Centre County offer an insightful example of the application of this acidification method using macroinvertebrate and water chemistry data. These sampling locations are all <25 mi^2, 2^{nd} or 3^{rd} order, with <25% palustrine wetlands and high gradient habitats, indicating that the samples are located in applicable settings to apply the wadeable freestone acidification assessment method (Table 4).

All sampling locations were Special Protection (HQ-CWF or EV) and had >50% base-poor geology. Boake Run (BOAKE) and Sterling Run (STERLING) had AML comprising 26.6 and 13.9% of their watershed area, respectively (Table 4, Figure 4). A passive AMD treatment facility was installed in Boake Run in 2005 upstream of the sampling location. There is no AMD treatment on Sterling Run. Pine Run (PINE_MOUTH) and Panther Run (PANTHER_CNTR) had 0% AML in their watersheds (Table 4, Figure 4).

Table 4. Watershed characteristics for Boake Run (BOAKE), Panther Run (PANTHER_CNTR), Pine Run (PINE_MOUTH), and Sterling Run (STERLING).

	BOAKE	STERLING	PINE_MOUTH	PANTHER_CNTR
Designated Use	HQ-CWF	HQ-CWF	HQ-CWF	EV
Drainage Area (mi^2)	3.1	4.7	3.8	6.7
Stream Order	2	2	3	3
% base-poor geology	81	55	75	72
% wetland	10.9	8.1	5.8	7
% AML	26.6	13.9	0	0
% forest	85.2	89.2	95.5	95.6

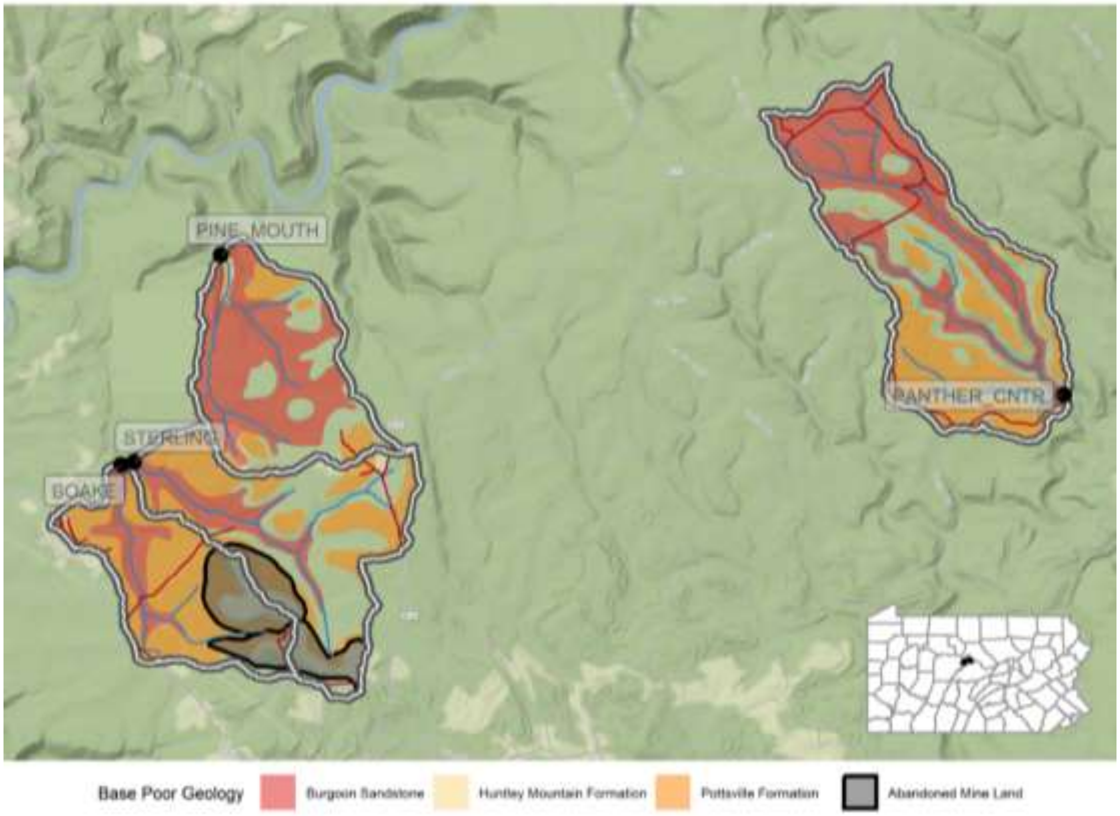

Figure 4. Boake Run (BOAKE), Sterling Run (STERLING), Pine Run (PINE_MOUTH), and Panther Run (PANTHER_CNTR) watersheds. Watershed boundaries are white, streams are blue segments, roads are maroon segments, abandoned mine lands (AML) are grey filled polygons, base-poor geology layers are filled accordingly and described in the legend. Monitoring locations are black points.

Data Collection and Results

Sample design followed guidance in *Stream and River Assessment Survey Design* (Shull and Arnold 2023b), where sampling locations represented waterbody condition and were located on small tributaries at an appropriate resolution to reflect influences of land cover, geology, and AML. At least one water chemistry sample was collected at each sampling location during period of biological sampling, following the *Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023a). Macroinvertebrate samples were collected following the *Wadeable Riffle Run Stream Macroinvertebrate Data Collection Protocol* (Shull 2017).

<u>Boake Run</u>

A macroinvertebrate sample collected at Boake Run in March 2019 (20190312-0815-jlorson) had an IBI score of 70.6 and an ATI score of 65.3 (Table 5). Two water chemistry samples were collected in April 2018 and March of 2019. Water quality was consistent with AMD settings, with t alkalinity, t Ca, t hardness, t Mg, d and t Mn, t Na, SpC, t SO_4, and t Zn concentrations higher than the 90th percentile of AD sites (Table 3). Median pH in the two samples was 6.2, reflecting treatment influence (Figure 5).

Sterling Run

A macroinvertebrate sample collected at Sterling Run in April 2018 (20180424-1330-mhoger) had an IBI score of 61.5 and an ATI score of 74.7 (Table 5). Two water chemistry samples were collected in April 2018 and March of 2019. Water quality was consistent with AMD settings with t Ca, hardness, t Mg, d and t Mn, SpC, t SO_4, and t Zn concentrations higher than the 90th percentile of AD sites and the pH below the 10th percentile of AD sites (Table 3, Figure 5).

Pine Run

A macroinvertebrate sample collected at Pine Run in March 2019 (20190311-1500-jlorson) had an IBI score of 61.7 and an ATI score of 59.4 (Table 5). One water chemistry sample was collected in March of 2019 showing conditions consistent with AD settings, with all parameters within 10^{th}-90^{th} percentiles of AD sites (Table 3, Figure 5).

Panther Run

A macroinvertebrate sample collected at Panther Run in March 2008 (20080318-1017-rryder) had an IBI score of 81.9 and an ATI score of 47.2 (Table 5). Two water chemistry samples were collected in August 2007 and March 2008. Water quality was consistent with non-acidified conditions. Alkalinity, t Ca, t Mg, pH, and SpC were higher than the 90th percentile of AD sites. Metals, including d and t Al, t Fe, d and t Mn, and t SO_4 were lower than 10th percentiles or within the 10th-90th percentile range of AD sites (Table 3, Figure 5).

Table 5. Benthic macroinvertebrate data from Boake Run (BOAKE), Panther Run (PANTHER_CNTR), Pine Run (PINE_MOUTH), and Sterling Run (STERLING). Taxonomic Serial Number (TSN) from the Integrated Taxonomic Information System (ITIS) and higher-level taxonomy are included with Acid Tolerance Values (ATV) and the number of individuals found at each site. Index of Biotic Integrity (IBI) and Acid Tolerance Index (ATI) scores are shown on the bottom rows.

TSN	Order	Family	Assessment ID	ATV	BOAKE	STERLING	PINE_MOUTH	PANTHER_CNTR
114244	Coleoptera	Elmidae	*Oulimnius*	2				34
114229	Coleoptera	Elmidae	*Promoresia*	3		6	2	1
127917	Diptera	Chironomidae	Chironomidae	4	11	12	49	15
136305	Diptera	Empididae	*Chelifera*	2				1
126703	Diptera	Simuliidae	*Prosimulium*	5	25	17	11	5
126774	Diptera	Simuliidae	*Simulium*	4		2		
119656	Diptera	Tipulidae	*Antocha*	2				1
121027	Diptera	Tipulidae	*Dicranota*	4			2	
100800	Ephemeroptera	Baetidae	*Baetis*	1			3	4
568550	Ephemeroptera	Baetidae	*Diphetor*	2				1
101360	Ephemeroptera	Ephemerellidae	*Dannella*				1	
101365	Ephemeroptera	Ephemerellidae	*Drunella*	1				5
101233	Ephemeroptera	Ephemerellidae	*Ephemerella*	2				11
101324	Ephemeroptera	Ephemerellidae	*Eurylophella*	5		1		1
100557	Ephemeroptera	Heptageniidae	*Cinygmula*	0				4
100626	Ephemeroptera	Heptageniidae	*Epeorus*	1			5	7
697957	Ephemeroptera	Heptageniidae	*Maccaffertium*	3				5
101187	Ephemeroptera	Leptophlebiidae	*Paraleptophlebia*	1				6
101766	Odonata	Gomphidae	*Lanthus*	3	1			2
102804	Plecoptera	Capniidae	*Paracapnia*	7	13			
103254	Plecoptera	Chloroperlidae	*Suwallia*		4			
102844	Plecoptera	Leuctridae	*Leuctra*	9	60	43	21	45
102540	Plecoptera	Nemouridae	*Amphinemura*	9	24	71	37	16
102622	Plecoptera	Nemouridae	*Ostrocerca*	9	3			
102489	Plecoptera	Peltoperlidae	*Peltoperla*	10			6	
102917	Plecoptera	Perlidae	*Acroneuria*	2	7			1
103166	Plecoptera	Perlodidae	*Diploperla*	2		2		
102995	Plecoptera	Perlodidae	*Isoperla*	3			3	
102471	Plecoptera	Pteronarcyidae	*Pteronarcys*	1				2

TSN	Order	Family	Assessment ID	ATV	BOAKE	STERLING	PINE_MOUTH	PANTHER_CNTR
102830	Plecoptera	Taeniopterygidae	*Oemopteryx*	7	2		1	
115399	Trichoptera	Hydropsychidae	*Diplectrona*	5	5	5	5	3
115453	Trichoptera	Hydropsychidae	*Hydropsyche*	1	13	1	3	
116794	Trichoptera	Lepidostomatidae	*Lepidostoma*	4		2		1
115319	Trichoptera	Philopotamidae	*Dolophilodes*	2	1			2
117044	Trichoptera	Polycentropodidae	*Polycentropus*	5		4		
115097	Trichoptera	Rhyacophilidae	*Rhyacophila*	5	7	2	13	8
116046	Trichoptera	Uenoidae	*Neophylax*	4				1
568832			Clitellata	5		1	2	25
			IBI Scores		70.6	61.5	61.7	81.9
			ATI Scores		65.3	74.7	59.4	47.2

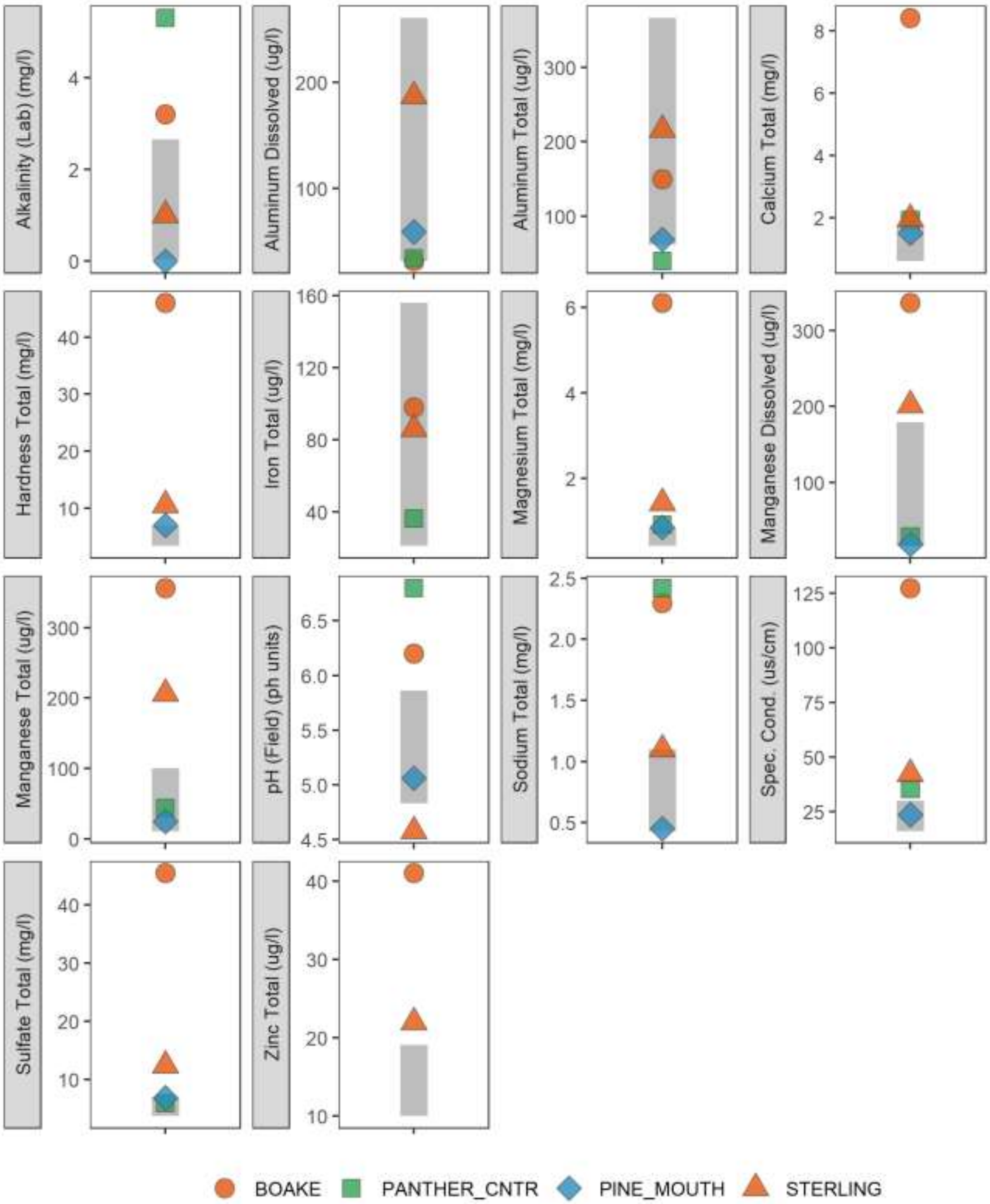

Figure 5. Boake Run (BOAKE), Panther Run (PANTHER_CNTR), Pine Run (PINE_MOUTH), and Sterling Run (STERLING) water chemistry results. Grey bars are 10th-90th percentile range of AD sites. Overlaid points are parameter-means for assessment sites.

Assessment and Source/Cause Decisions
The macroinvertebrate sample at Pine Run had scores exceeding ATI (>53) and IBI (<63) thresholds, indicating impairment using both the wadeable freestone acidification assessment method and the *Wadeable Freestone Riffle-Run Stream Macroinvertebrate Assessment Method* (Shull 2023). Water chemistry was consistent with AD settings with low pH and low concentrations of ions, sulfate, metals, SpC, and hardness. All chemical parameters were within 10^{th}-90^{th} percentiles of AD sites. Due to the biological metrics and the available chemical data, the source of impairment was Atmospheric Deposition and the cause was pH, Low.

The macroinvertebrate sample at Boake Run had a supporting IBI score (>63), but an impaired ATI score (>53). The macroinvertebrate sample at Sterling Run had an impaired IBI score (<63) and an impaired ATI score (>53). Independent applicability requires that assessment methods be applied independently to make assessment decisions, which results in impairments at both Boake and Sterling Run. Water chemistry at both locations was consistent with surface water acidification with AMD influence. Hardness, SO_4, metals and SpC were elevated. The AML in the watershed was contributing to elevated concentrations of metals, ions, and hardness at the site beyond what would be considered AD only. Boake Run had elevated alkalinity, pH, Ca, and Mg, reflecting treatment influence. Due to the evidence from water chemistry data and the presence of AML in the watersheds, Acid Mine Drainage was a source of impairment. Atmospheric deposition was a contributing source as well, due to the high percentage of base-poor geology in the watershed and the evidence that the neighboring watershed (Pine Run) had no AML but was still impaired due to the ATI score with an AD source. Due to the biological metrics and the available chemical data, the sources of impairment for Boake Run and Sterling Run were Acid Mine Drainage and Atmospheric Deposition and the cause was pH, Low.

Both IBI and ATI scores were supporting at PANTHER_CNTR. The water quality at PANTHER_CNTR was consistent with non-acidified conditions. Alkalinity, base cations and pH were elevated when compared to the 90^{th} percentiles of AD sites, and metals were present in low concentrations. After examining the available data, this site was supporting per the acidification assessment method.

Although not discussed, since these were Special Protection waters, baseline ATI scores should also be investigated for changes of ≥8 points to complete the Special Protection assessment.

<u>ASSESSMENT EXAMPLE – FISH</u>

Applicable Setting Determination
Three watersheds in southern Tioga County offer an insightful example of the application of this method using fish assemblage and water chemistry data. These sampling locations are all <25 mi^2, 2^{nd} or 3^{rd} order, with <25% palustrine wetlands and high gradient habitats, indicating that the samples are located in applicable settings to apply the wadeable freestone acidification assessment method (Table 6).

Rock Run (ROCKRUN_1.2) and Unnamed Tributary to (UNT) Babb Creek (UNT_BABB_0.1) were designated Cold Water Fishes (CWF) and Nickel Run (NICKEL_0.1) was Special Protection (EV). All sampling locations had >50% base-poor geology. Rock Run had AML comprising 9% of watershed area. AMD treatment was established in the early 2000s and discharges into the sole western tributary. Nickel Run and UNT Babb Creek had 0% AML in their watersheds (Table 6, Figure 6).

Table 6. Watershed characteristics for Rock Run (ROCKRUN_1.2), Nickel Run (NICKEL_0.1), and an unnamed tributary to Babb Creek (UNT_BABB_0.1).

	ROCKRUN_1.2	**NICKEL_0.1**	**UNT_BABB_0.1**
Designated Use	CWF	EV	CWF
Drainage Area (mi^2)	4.1	3.2	3.2
Stream Order	2	2	2
% base-poor geology	100	100	100
% wetland	12.6	9.5	12.9
% AML	9	0	0
% forest	83.8	94.2	90.5

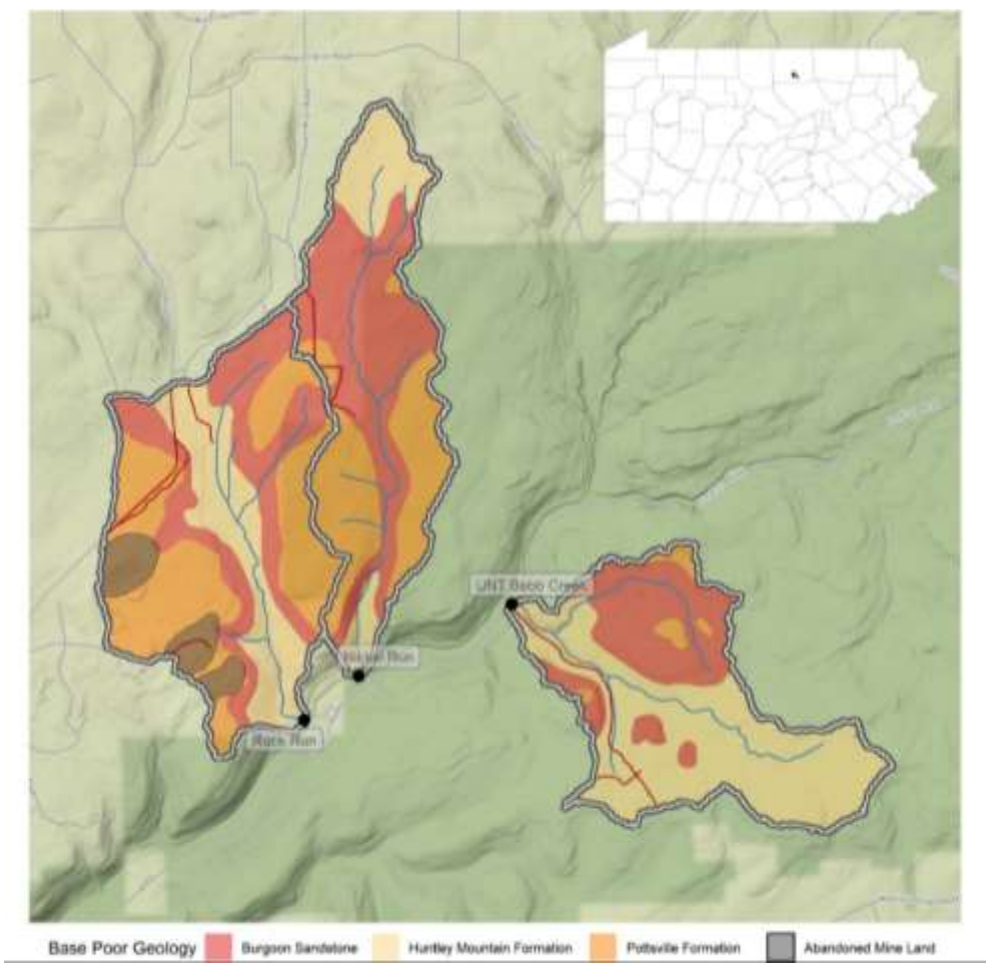

Figure 6. Nickel Run (NICKEL_0.1), Rock Run (ROCKRUN_1.2), and an unnamed tributary to Babb Creek (UNT_BABB_0.1) watersheds. Watershed boundaries are white, streams are blue segments, roads are maroon segments, abandoned mine lands (AML) are grey filled polygons, base-poor

geology layers are filled accordingly and described in the legend. Monitoring locations are black points.

Data Collection and Results

Sample design followed guidance in *Stream and River Assessment Survey Design* (Shull and Arnold 2023b), where sampling locations represented waterbody condition and were located on small tributaries at an appropriate resolution to reflect influences of land cover, geology, and AML. At least one water chemistry sample was collected at each sampling location during period of biological sampling, following the *Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023a). Fish community samples were collected following the *Fish Data Collection Protocol* (Wertz and Arnold 2023).

Nickel Run

A fish sample collected in July 2022 (20220707-0832-dushull) had a TFI score of 2.8. A total of 124 individuals were collected during the survey with 38.7% $\boldsymbol{FishGenera_{AcidIntol}}$ (Table 7). Eight water chemistry samples were collected from January – November 2022. Water quality was consistent with non-acidified conditions. Alkalinity, t Ca, hardness, and pH were higher than the 90^{th} percentile of AD sites and acidity lower than the 10^{th} percentile. Metals, including d and t Al, t Fe, d and t Mn, and t SO_4 were lower than 10th percentiles or within the 10th-90th percentile range of AD sites (Table 3, Figure 7).

Rock Run

A fish sample collected in July 2022 (20220707-1006-mshank) had a TFI score of 4.3. A total of 41 individuals were collected during the survey with 0% $\boldsymbol{FishGenera_{AcidIntol}}$ (Table 7). Eight water chemistry samples were collected from January – November 2022. Water quality was consistent with AMD settings, with t alkalinity, t Ca, TDS, hardness, t Mg, d and t Mn, t Na, pH, SpC, t SO_4, and t Zn concentrations higher than the 90th percentile of AD sites. Median pH was 6.2, reflecting treatment influence (Table 3, Figure 7).

UNT Babb Creek

A fish sample collected in July 2022 (20220706-1435-mshank) had a supporting TFI score of 2.0. A total of 39 individuals were collected during the survey with 0% $\boldsymbol{FishGenera_{AcidIntol}}$ (Table 7). Eight water chemistry samples were collected from January – November 2022 showing conditions consistent with AD settings, with all parameters within 10^{th}-90^{th} percentiles except t Zn (Table 3, Figure 7).

Table 7. Fish data from Nickel Run (NICKEL_0.1), Rock Run (ROCKRUN_1.2), and an unnamed tributary to Babb Creek (UNT_BABB_0.1). Taxonomic Serial Number (TSN) from the Integrated Taxonomic Information System (ITIS) are included with the number of individuals of each species found at each site and relative abundance (in parentheses). The bottom three rows include Thermal Fish Index (TFI) score, total number of individuals captured in the sample (Total Indiv.) and the sum of relative abundance of *Cottus, Etheostoma, Clinostomus,* and *Oncorhynchus* individuals (***FishGenera**$_{AcidIntol}$*).

TSN	Family	Genus	Scientific Name	Common Name	NICKEL_0.1	ROCKRUN _1.2	UNT_BABB_0.1
161997	Salmonidae	*Salmo*	*Salmo trutta*	brown trout	7 (5.6 %)		
162003	Salmonidae	*Salvelinus*	*Salvelinus fontinalis*	brook trout	47 (37.9 %)	17 (41.5 %)	39 (100 %)
167232	Cottidae	*Cottus*	*Cottus cognatus*	slimy sculpin	48 (38.7 %)		
163382	Cyprinidae	*Rhinichthys*	*Rhinichthys atratulus*	eastern blacknose dace	9 (7.3 %)	2 (4.9 %)	
163384	Cyprinidae	*Rhinichthys*	*Rhinichthys cataractae*	longnose dace	13 (10.5 %)		
163873	Cyprinidae	*Margariscus*	*Margariscus margarita*	pearl dace		1 (2.4 %)	
163376	Cyprinidae	*Semotilus*	*Semotilus atromaculatus*	creek chub		21 (51.2 %)	
				TFI	2.8	4.3	2.0
				*Total Indiv.	124	41	39
				****FishGenera**$_{AcidIntol}$*	38.7%	0%	0%
				Acidification Metric Results*	Both Above	Both Below	Both Below

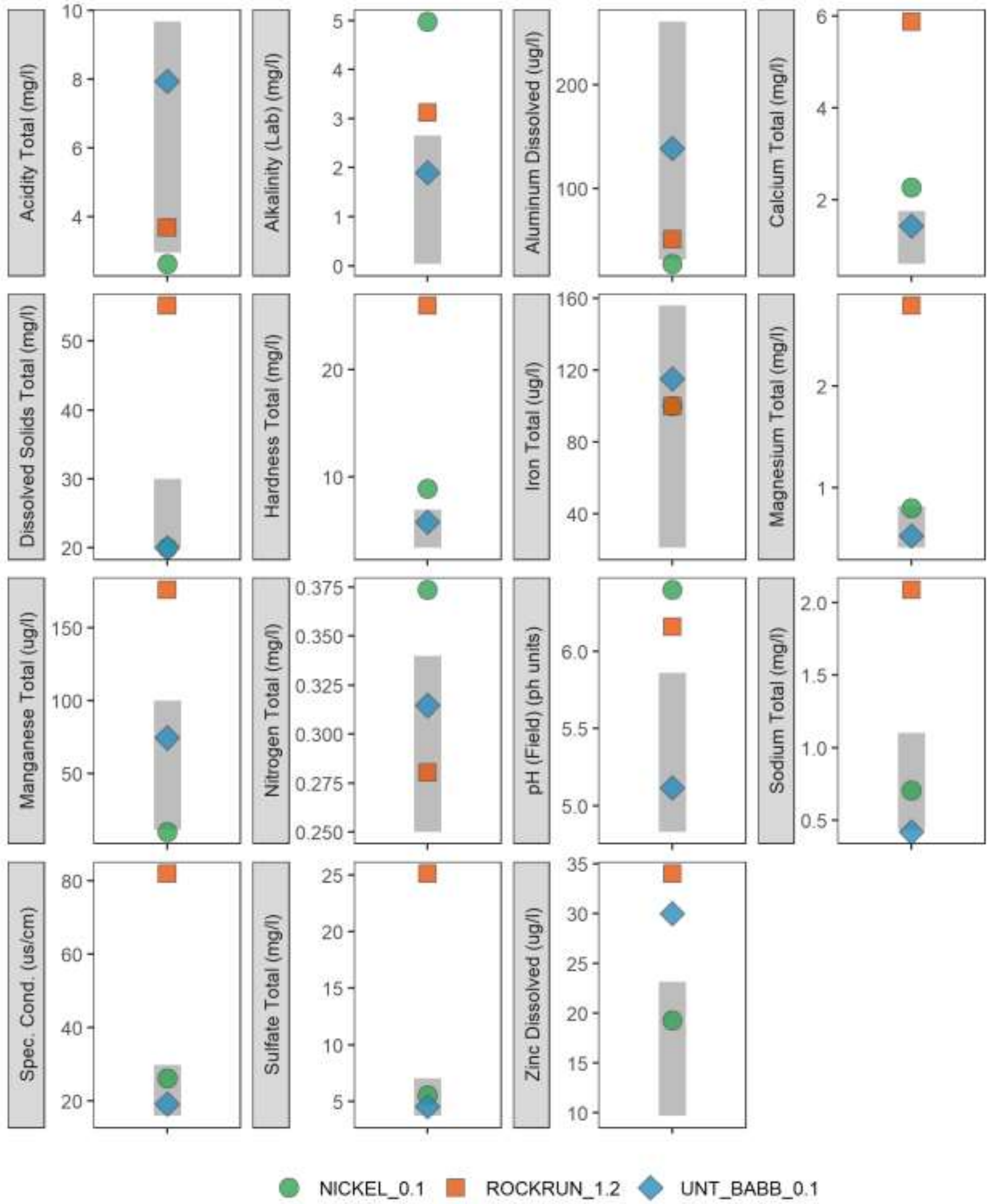

Figure 7. Nickel Run (NICKEL_0.1), Rock Run (ROCKRUN_1.2), and unnamed tributary to Babb Creek (UNT_BABB_0.1) water chemistry results. Grey bars are 10th-90th percentile range of AD sites. Overlaid points are parameter-means for assessment sites.

Assessment and Source and Cause Decisions

Rock Run, Nickel Run, and UNT Babb Creek had TFI scores <4.8, which is the impairment threshold for their drainage area group (DAG). However, due to <50 individuals collected at Rock Run and UNT

Babb Creek, an investigation into representativeness and toxic or sterile conditions would be required by the *Stream Fish Assemblage Assessment Method* (Wertz 2021). Rock Run and UNT Babb Creek have abundance (total individuals < 50) and composition (***FishGenera**$_{AcidIntol}$* <10%) metrics below wadeable freestone acidification assessment method thresholds, indicating impairment.

The water chemistry at UNT Babb Creek was consistent with AD settings with low pH and low concentrations of ions, sulfate, metals, SpC, and hardness. All chemical parameters were within 10^{th}-90^{th} percentiles of AD sites, except for t Zn. Due to the fish community and the available chemical data, the site was impaired with source Atmospheric Deposition and cause pH, Low.

Water chemistry at Rock Run was consistent with surface water acidification with AMD influence. Alkalinity, hardness, Ca, Mg, SO_4, metals, and SpC were elevated, indicating the AML in the watershed was contributing to elevated concentrations of at the site beyond what would be considered AD only. Elevated metals coupled with elevated alkalinity, hardness, pH, Ca, and Mg reflected treatment influence. Due to the evidence from water chemistry data and the presence of AML in the watersheds, the source of impairment was acid mine drainage (AMD) with cause pH, Low. Atmospheric deposition was a contributing source as well, due to the high percentage of base-poor geology in the watershed and the evidence that a proximate watershed (UNT Babb Creek) had no AML but was still impaired due to wadeable freestone acidification method fish metrics below thresholds and AD source. Due to the biological metrics and the available chemical data, the sources of impairment for Rock Run were Acid Mine Drainage and Atmospheric Deposition and the cause was pH, Low.

Both TFI scores and acidification fish metrics were supporting at Nickel Run. Water quality was consistent with non-acidified conditions. Alkalinity, base cations and pH were elevated when compared to the 90^{th} percentiles of AD sites, and metals were present in low concentrations. After examining the available data, this site was supporting per the acidification assessment method.

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