

**Project Name** | Brown's Creek Biological Assessments

**Date** | 04/03/2026

**To / Contact info** | BCWD Board of Managers

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**From / Contact info** | Mike Majeski, Conservation Biologist

**Regarding** | Macroinvertebrate Data Summary\_2016-2025

## Executive Summary

Macroinvertebrate monitoring conducted at three stream sites from 2016 to 2025 indicates a clear and sustained improvement in stream health over the course of the monitoring period. Index of Biotic Integrity (IBI) scores — a standardized measure of aquatic community health based on the types and abundance of macroinvertebrates present — have trended upward across all three sites, with most scores falling between the General Use and Exceptional Use thresholds established for the Southern Coldwater Streams region.

The improvement has been most pronounced at the Gorge site, where IBI scores have consistently exceeded the Exceptional Use threshold since 2019. This trend is reflected in the broader dataset as well — of the 20 samples that have scored above the Exceptional Use threshold across all sites and years, 17 have occurred since 2019, including all three fall 2019 samples. Taken together, these results indicate that the stream's macroinvertebrate community is recovering.

## Background

The BCWD has been conducting routine fish and macroinvertebrate assessments since 2016 to monitor changes in the biological community of Brown's Creek following implementation of numerous water quality projects in the watershed (see implementation activity under Stream Management, Goal A of the 2017-2026 Watershed Management Plan). The goals of BCWD's routine fish and macroinvertebrate assessments are to develop a more robust understanding of the variability of species composition over time and to develop a long-term trend analysis of changes to the biological community in Brown's Creek in response to on-going water quality projects implemented in the watershed. Macroinvertebrate assessments have been conducted annually as populations and species diversity can change quickly due to changes in their environment, in part due to their short life spans and sensitivities to changes in water quality. Conversely, fish have longer lifespans and populations are generally slower to respond to changes in their environment compared to macroinvertebrates. The DNR conducts routine fish surveys on a 3-year basis in Brown's Creek with the last survey completed in Fall 2024.

The Minnesota Pollution Control Agency (MPCA) has been using fish and macroinvertebrate data to assess the watershed's water quality impairments and designated uses as part of their long-term Intensive Watershed Monitoring Plan. As part of MPCA's biological assessment, fish and macroinvertebrate-based indices of biological integrity (IBI) have been developed to track long-term trends in the biological community of each watershed studied in Minnesota. Fish and macroinvertebrate IBI's are based on the number and diversity of fish and macroinvertebrate species present in a stream compared to what the stream is expected to support. The following is a summary of macroinvertebrate data collected from 2016-2025.

## **2025 Macroinvertebrate Assessment**

Macroinvertebrates were sampled from three sites along Brown's Creek including the Headwaters, Middle Reach, and Gorge (Figure 1). Starting in 2023, macroinvertebrate sampling has been only conducted in the fall (September) based on input and recommendations from MPCA staff. The fall season is the recommended sampling period since the overall macroinvertebrate community is better represented in the fall (e.g., more species are present in the fall compared to the spring).

Macroinvertebrate specimens were sent to RMB Environmental Laboratories for taxonomic identification to the genus level, and a subsequent report was completed summarizing the macroinvertebrate IBI scores and results from the 2016-2025 surveys (Appendix A). Findings and trends from the macroinvertebrate surveys are summarized below.

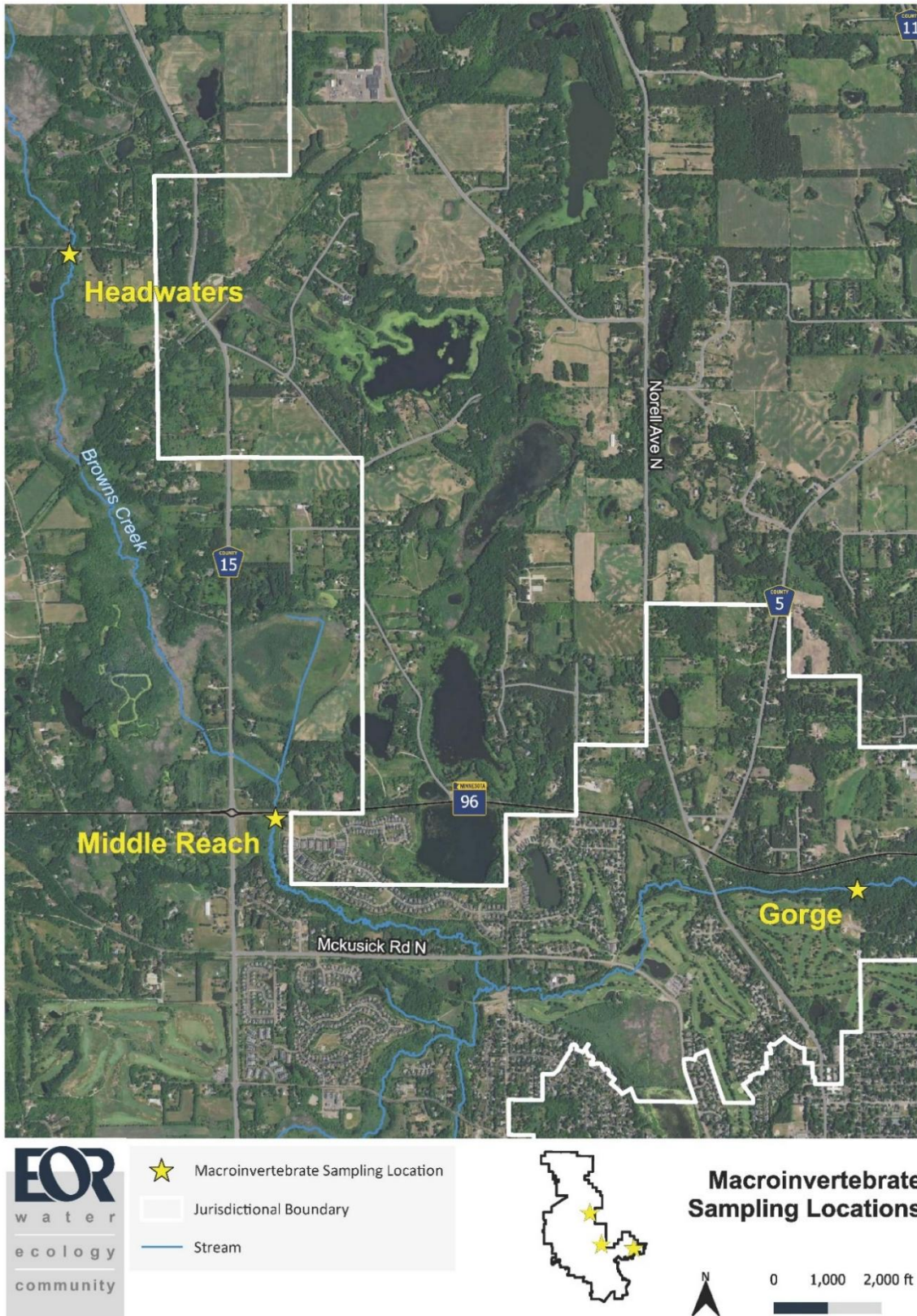
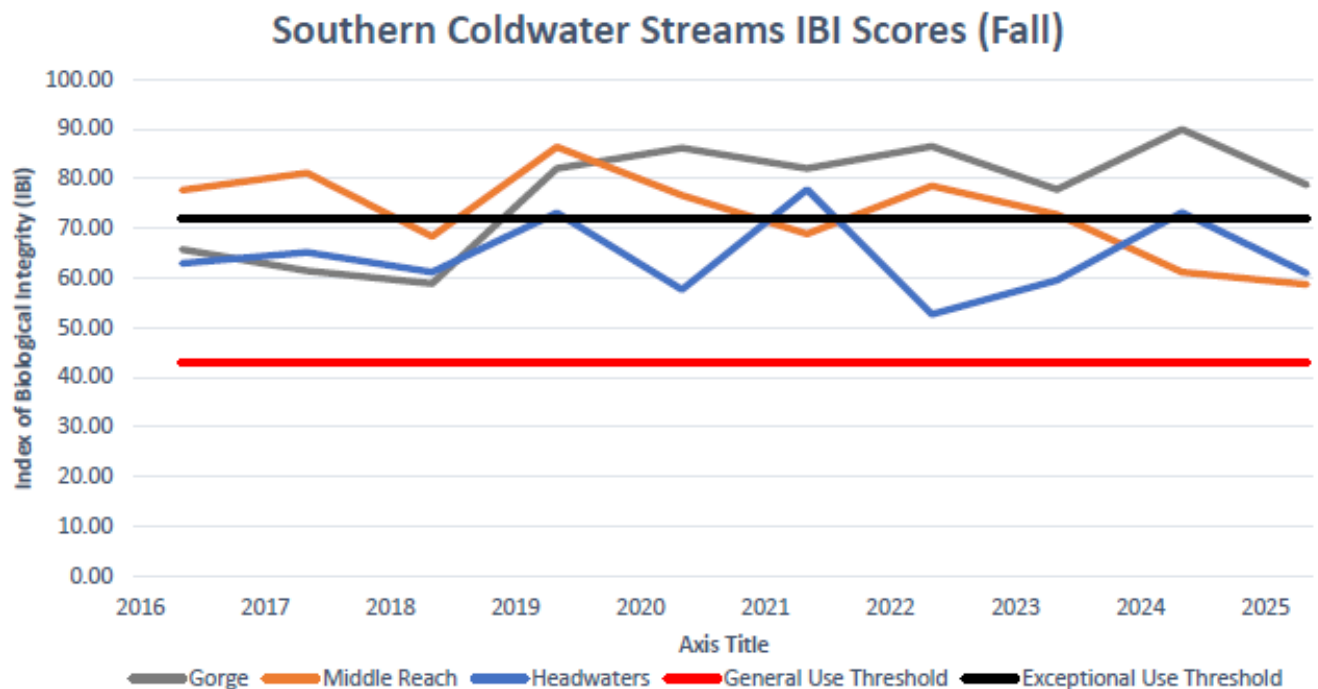


Figure 1. Macroinvertebrate sampling locations in the BCWD, 2016-2025.

## Findings & Trends

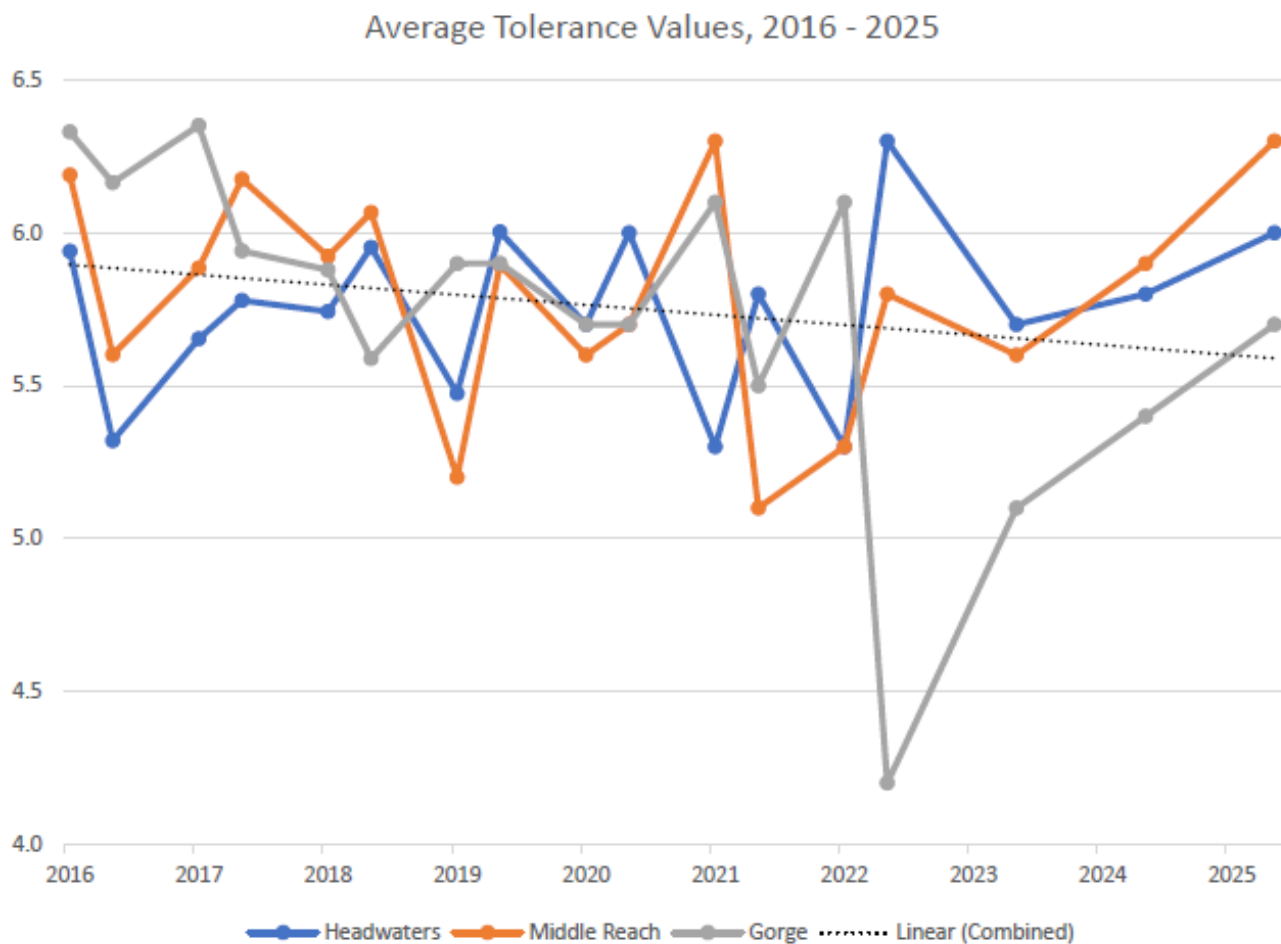
- Data collected from 2016-2025 indicates an overall upward (improving) trend in stream health and macroinvertebrate community quality.
- The calculated IBI scores from all 3 sites from 2016-2025 indicate an improving macroinvertebrate community since 2016, with most macroinvertebrate IBI scores occurring between the General Use and Exceptional Use thresholds for the Southern Coldwater Streams region (Figure 2). In particular, the Gorge IBI scores have improved the most during the study and have remained above the Exceptional Use threshold since 2019. Of the 20 samples that have scored above the Exceptional Use Threshold over the course of the monitoring project, 17 of those samples have occurred since 2019. Most notably, all three fall 2019 samples were above the Exceptional Use Threshold.



**Figure 2. Fall season IBI scores from Brown's Creek and associated General Use and Exceptional Use thresholds.**  
Source: RMB Macroinvertebrate Stream Monitoring Assessment 2016-2025 (Figure 6, Appendix A).

- The total number of taxa (a unique group of organisms) sampled from 2016-2025 indicates a diversity of macroinvertebrates present across all 3 sites (161 unique taxa to date), with the three most dominant taxa having a medium-level tolerance to pollution: black flies, freshwater shrimp "scuds", and non-biting midges. However, good numbers of intolerant taxa (groups intolerant of pollution) are also present which indicates the stream provides ample habitat and water quality to support sensitive taxa such as stoneflies and "saddle casemaker" caddisflies.

- Perlodid stoneflies have been collected every year from the Gorge site, indicating the creek provides ample habitat and high oxygen levels to support this pollution intolerant group. Perlodid stoneflies were also collected from the Middle Reach in 2020 and from 2022-2024.
- The average pollution tolerance value has decreased since 2016, indicating the creek is supporting a greater number of taxa that are considered intolerant to pollution (Figure 3). This trend is also reflective in the population size of intolerant taxa, with the total number of pollution intolerant taxa generally increasing since 2016 (Figure 4). Pollution intolerant taxa are present in good numbers at all 3 sampling sites and suggest Brown’s Creek is providing suitable habitat and water quality at these monitoring locations. However, the increase in the Middle Reach average pollution tolerance value for 2025 is attributed to a significant increase in water temperature from a large beaver dam that occurred throughout the summer season upstream of the sample site. The warmer water measured in 2025 likely favored pollution tolerant taxa that dominated the Middle Reach sample in 2025 and resulted in a decline in pollution intolerant taxa (Figure 4).



**Figure 3. Average pollution tolerance values for Brown’s Creek macroinvertebrates from 2016-2025. Source: RMB Macroinvertebrate Stream Monitoring Assessment 2016-2025 (Figure 4, Appendix A).**

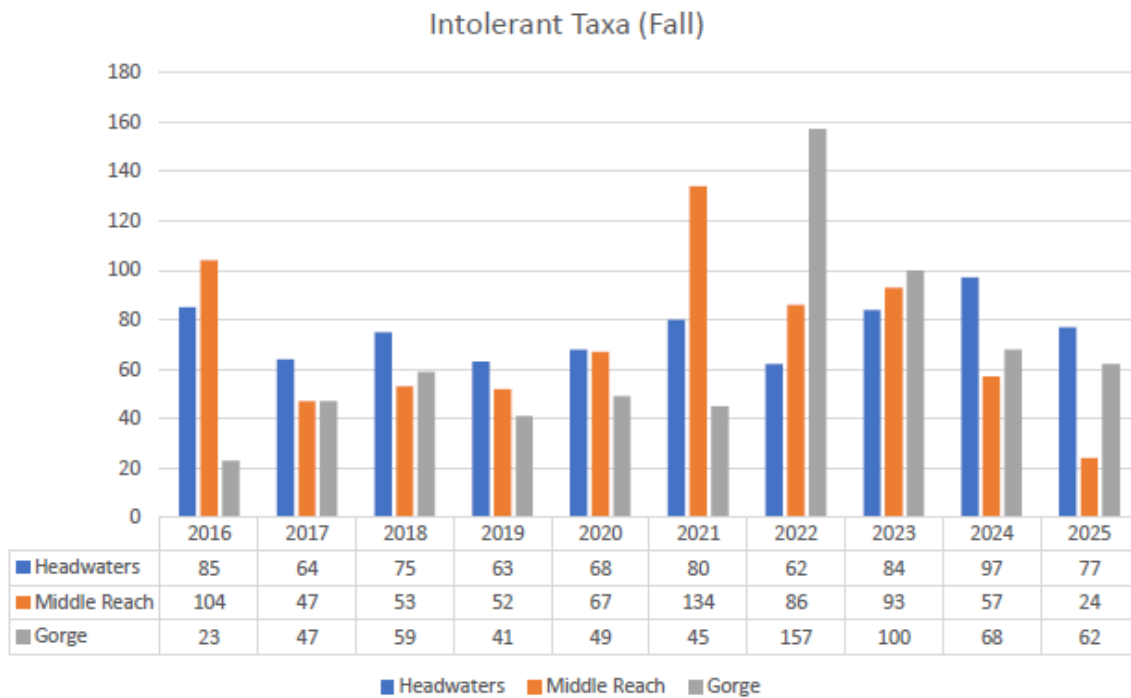


Figure 4. Comparison of total numbers of intolerant taxa collected in Brown's Creek from 2016-2025 (Fall samples only). Source: RMB Macroinvertebrate Stream Monitoring Assessment 2016-2025 (Figure 9, Appendix A).

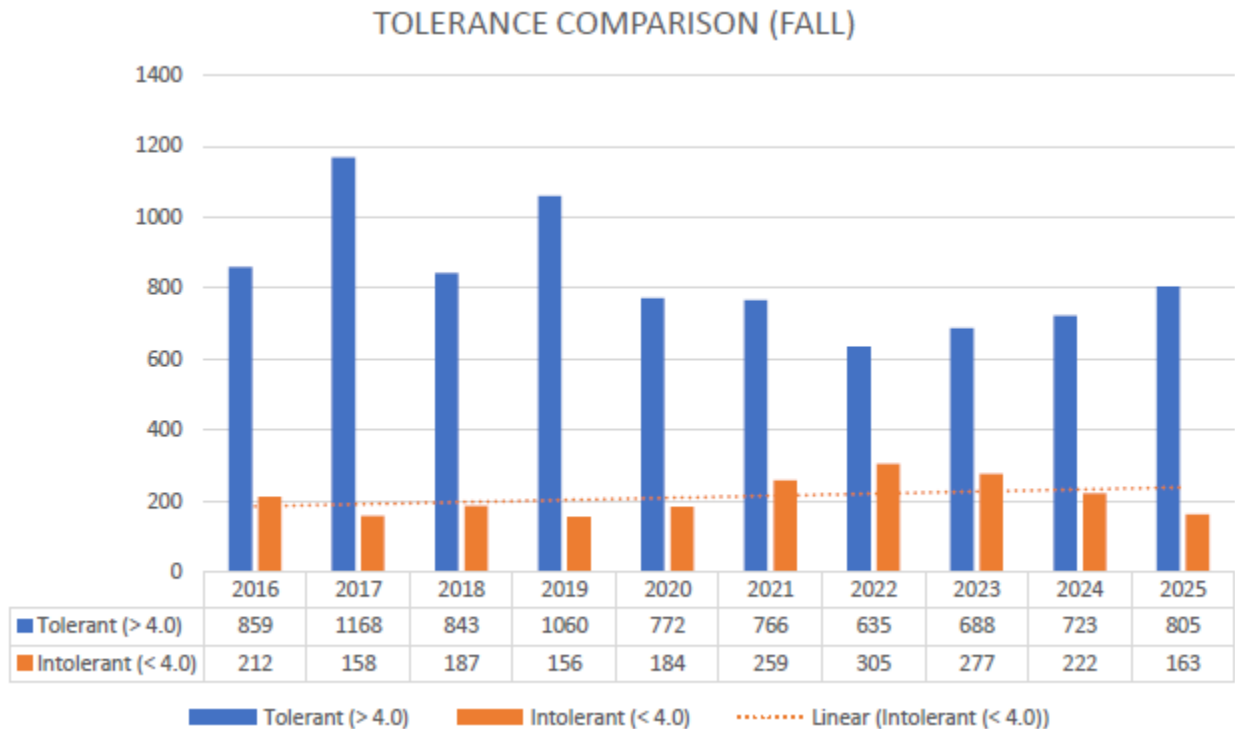


Figure 5. Comparison of tolerant and intolerant taxa in Brown's Creek. Source: RMB Macroinvertebrate Stream Monitoring Assessment 2016-2025 (Figure 11, Appendix A).

## **Discussion**

The section of Brown's Creek between Manning Avenue and the St. Croix River is designated as a Class 2A stream, supporting coldwater aquatic life and habitat. The ideal fish community (or highest fish IBI score) for a Class 2A coldwater stream would be comprised solely of species intolerant to warm water such as brook trout and sculpin. Since both of these species are absent from Brown's Creek, the fish IBI scores have been historically low despite supporting a naturally reproducing population of brown trout. It is unknown when native brook trout became extirpated from Brown's Creek, but DNR fish survey records indicate introduced "East Coast" brook trout were stocked in the creek sometime in the 1970's. The introduced brook trout did not fair well in Brown's Creek which led to the stocking of non-native brown trout to support recreational trout angling in the area. However, the creek has slowly cooled over the last decade following implementation of several water quality and stream habitat projects in the watershed. The creek now provides a thermal regime that may support Minnesota native strain brook trout which are genetically unique to Minnesota streams. If the Minnesota Department of Natural Resources decides to reintroduce native strain brook trout to Brown's Creek, and the brook trout begin to naturally reproduce, fish IBI scores would improve significantly and may result in delisting Brown's Creek for the existing Aquatic Life impairment.

## **Appendix A**

RMB Report: Macroinvertebrate Stream Monitoring Assessment 2016-2025

# Macroinvertebrate Stream Monitoring Assessment 2016 – 2025

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Emmons & Olivier  
Resources, Inc.

March 19th, 2026

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RMB Environmental Laboratories  
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Report Date: March 19<sup>th</sup>, 2026

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taxon quality control verification

Joel Chirhart, Minnesota Pollution Control Agency: IBI calculations

Kaira Kamke: Report

Moriya Rufer: Report

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## Introduction

Macroinvertebrates provide a valuable insight into the health of a stream ecosystem since most taxa require specific conditions to survive and thrive. Stream parameters like temperature, flow speed, substrate type, dissolved oxygen, and pollution inputs can all impact which invertebrates will be found at a site. Evaluating the invertebrate community in a stream or river can reveal impacts to the aquatic ecosystem and trends in the water quality.

From 2016 – 2022, aquatic macroinvertebrates were collected in May or June and September from Brown’s Creek in Washington County, Minnesota. In 2023 – 2025, aquatic macroinvertebrates were collected in September only. The Minnesota Pollution Control Agency (MPCA) Index of Biotic Integrity (IBI) was calculated for all stream sites to assess the water quality and compare sites. Samples were collected along the stream reach at the Headwaters, Middle Reach, and Gorge sites to evaluate how the quality changes along the gradient (Figure 1). Brown’s Creek is located within the Southern Coldwater Streams invertebrate class (Figure 2). Samples were repeated each year beginning in 2015 to evaluate changes over time. The collection of this data is essential for compiling a baseline dataset of invertebrates found in this region, which can be used for assessments of impacts or future restoration projects on this stream.

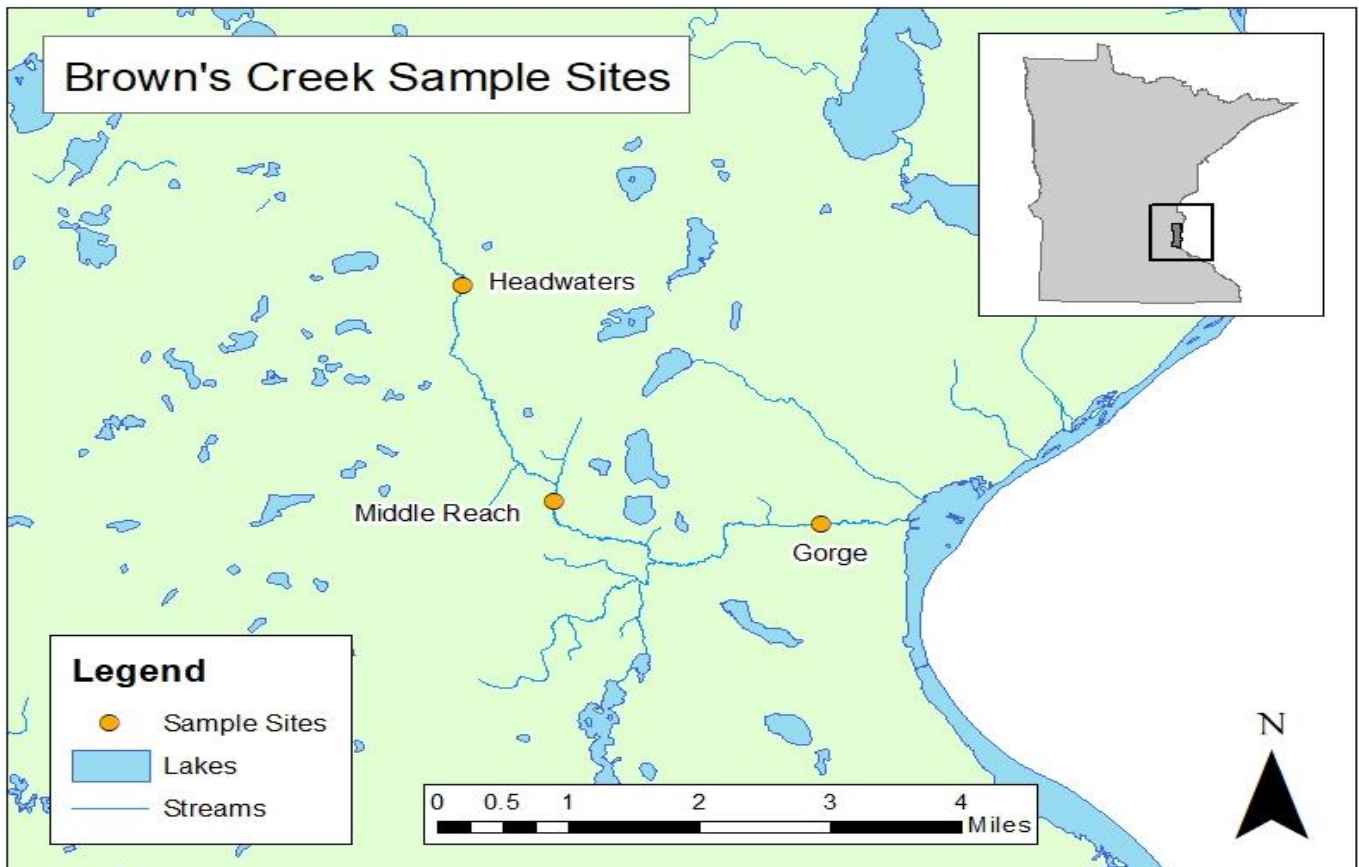


Figure 1: Macroinvertebrate monitoring sites in Brown’s Creek, 2016-2025

## Methods

### *Sample Collection*

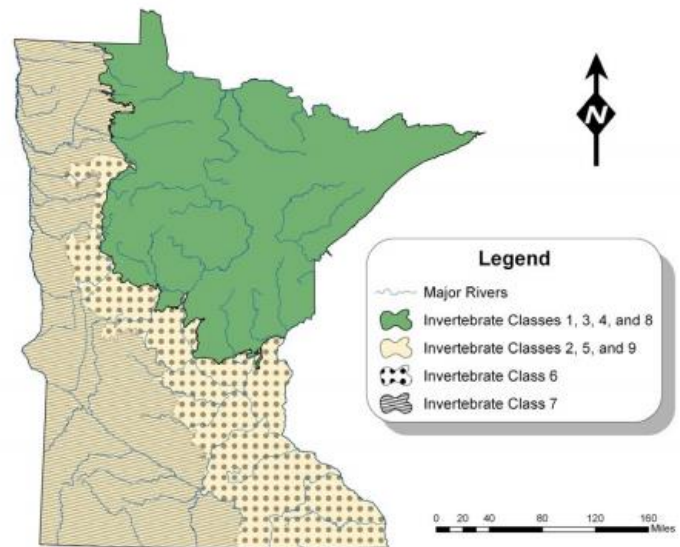
The aquatic macroinvertebrate samples collected from 2016 – 2025 were located at the Headwaters, Middle Reach, and Gorge sites of Brown’s Creek. Samples were collected with a D-frame net following the MPCA’s Standard Operating Procedure (SOP) for multi-habitat collection of stream invertebrates (MPCA). They were then preserved and delivered to RMB Environmental Laboratories, Inc. (RMBEL) in Detroit Lakes, MN for laboratory processing and data analysis.

### *Laboratory Processing*

The macroinvertebrate samples were processed following MPCA methods, including sorting random subsamples to a target specimen count of 300. All taxa were enumerated and identified to genus level, with leeches and snails identified to species where possible. Representative taxa were retained in a project collection for internal quality control. Subsample picking and taxa identifications were both held to 95% efficiency in internal quality control checks.

### *Data Management and Assessment*

The final data for each sample was entered into a spreadsheet and sent to Joel Chirhart at the MPCA to run the IBI database calculations. RMBEL staff used the macroinvertebrate community data to calculate general invertebrate metrics to accompany the IBI values and facilitate comparison among sites along the stream gradient and across years. Sites were mapped in ArcMap to regionally compare the samples, which are within the Southern Coldwater Streams invertebrate class (Class 9). These classes are derived from the Minnesota Department of Natural Resources (DNR) Ecological Classification System provinces and were developed based on major climate zones, native vegetation, and biomes.



[Figure 2: Map of Minnesota Pollution Control Agency invertebrate classes \(MPCA\)](#)

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## Results

### *Macroinvertebrate Metrics*

Macroinvertebrate metrics can provide a general overview of the health of a stream ecosystem relating to which taxa are dominant in a sample and how many taxa are intolerant to pollution impacts. Overall taxa richness is a common metric for water quality, since unimpacted stream systems typically show much more diversity than those with heavy impacts. The taxa richness values in this report include only unique taxa, and specimens that are immature or damaged and left at a higher taxonomic level were omitted from the metric. This may present some discrepancies from previous reports sent, in which all taxa were included in the richness values, regardless of whether they were unique to the rest of the community composition. Evaluating certain taxa groups that generally prefer specific conditions can give an idea of whether the stream quality is higher or lower than other sites. These include Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies), which typically are found in unpolluted waters, as well as Chironomidae (midges) which tend to dominate in highly impacted sites. Additionally, the presence of taxa that are intolerant to pollution can indicate higher quality waters. These metrics are explained in Table 1; they have been calculated for all the samples throughout this project and are listed in Tables 2 – 7.

Table 1. Explanations of the macroinvertebrate metrics

| <b>Metric</b>               | <b>Explanation</b>  | <b>Response</b>   |
|-----------------------------|---|---|
| <b>Taxon Richness</b>       | <b>The total number of taxa found in the sample (genus level, family level for Chironomidae)</b>  | <b>Higher numbers indicate better water quality and habitat quality</b> |
| <b>EPT Richness</b>         | <b>The total number of Ephemeroptera (mayflies), Plecoptera (Stoneflies), and Trichoptera (caddisflies) in the sample. These taxa are considered generally intolerant to pollution.</b> | <b>Higher numbers indicate better water quality and habitat quality</b> |
| <b>Plecoptera Richness</b>  | <b>The total number of Plecoptera (stoneflies) taxa in the sample. Plecoptera are intolerant to pollution and are clean water indicators.</b>   | <b>Higher numbers indicate better water quality and habitat quality</b> |
| <b>Percent Chironomidae</b> | <b>Generally, the more chironomids in a sample, the more impacted the site is.</b>  | <b>Lower numbers indicate better water quality and habitat quality</b>  |
| <b>Average Tolerance</b>    | <b>The average tolerance value of all the taxa in the sample on a 0-10 scale, with 0 being intolerant to organic pollution and 10 being tolerant to organic pollution</b>               | <b>Lower numbers indicate better water quality and habitat quality</b>  |
| <b>Intolerance</b>          | <b>Number of taxa with tolerance values less than or equal to 4</b>   | <b>Higher numbers indicate better water quality and habitat quality</b> |

## 2016 Results

In 2016, the samples had a total of 55 different taxa found. The taxon richness values for each sample were mostly above 20, which represents a diverse community of invertebrates (Table 2). Only the Gorge sample from September showed a lower taxa richness than the other samples, with only 15 unique taxa. The EPT richness was also found to be high, with every sample having at least 3 different taxa from one of those insect groups. The Plecoptera richness was comparable to the 2015 samples, with only the Gorge site having stoneflies present, but they were found in both the May and September samples this year.

The percent Chironomidae was low again for the samples in 2016, with the highest only reaching 14.2%. However, the average tolerance values were slightly above 5.0 again, indicating the domination of tolerant taxa in the samples. Each of the samples displayed intolerant taxa, so each site does not show the high impact levels that would prevent those species from occurring there.

Table 2. Metrics for each sample site in 2016

| Site                | Taxon Richness | EPT Richness | Plecoptera Richness | Percent Chironomidae | Average Tolerance | Intolerance |
|---------------------|----------------|--------------|---------------------|----------------------|-------------------|-------------|
| <b>Headwaters</b>   | <b>36</b>      | <b>8</b>     | <b>0</b>            | <b>6.0%</b>          | <b>5.6</b>        | <b>3</b>    |
| May                 | 20             | 4            | 0                   | 7.6%                 | 5.9               | 1           |
| September           | 28             | 6            | 0                   | 4.5%                 | 5.3               | 3           |
| <b>Middle Reach</b> | <b>36</b>      | <b>7</b>     | <b>0</b>            | <b>6.8%</b>          | <b>5.8</b>        | <b>3</b>    |
| May                 | 20             | 3            | 0                   | 14.2%                | 6.2               | 1           |
| September           | 23             | 5            | 0                   | 1.4%                 | 5.6               | 3           |
| <b>Gorge</b>        | <b>27</b>      | <b>3</b>     | <b>1</b>            | <b>11.6%</b>         | <b>6.2</b>        | <b>2</b>    |
| May                 | 21             | 3            | 1                   | 12.2%                | 6.3               | 1           |
| September           | 15             | 3            | 1                   | 1.1%                 | 6.2               | 1           |

## 2017 Results

The macroinvertebrate samples taken in 2017 again showed high-quality water overall, with 60 unique taxa found across all the sites (Table 3). The taxon richness was higher for most of the samples than in previous years, and all sites had several EPT taxa present. Plecoptera were again found only at the Gorge site, but in both the spring and fall samples. The Chironomidae proportion was higher in some of the sites this year than in previous years, with the most being present in the Headwaters sample from May. However, most of the midge taxa found were *Diamesa* and *Parametriocnemus*, which both have moderate tolerance values of 5.0 and 5.2, respectively. Midges that dominate in heavily impacted streams tend to have tolerance values much higher than those found in this sample. The average tolerance values for the samples were like previous years in the 5.5 – 6.5 range, and each site had some intolerant taxa found.

Table 3. Metrics for each sample site in 2017

| Site                | Taxon Richness | EPT Richness | Plecoptera Richness | Percent Chironomidae | Average Tolerance | Intolerance |
|---------------------|----------------|--------------|---------------------|----------------------|-------------------|-------------|
| <b>Headwaters</b>   | <b>31</b>      | <b>6</b>     | <b>0</b>            | <b>33.2%</b>         | <b>5.7</b>        | <b>4</b>    |
| May                 | 18             | 3            | 0                   | 51.5%                | 5.7               | 2           |
| September           | 23             | 4            | 0                   | 4.8%                 | 5.8               | 3           |
| <b>Middle Reach</b> | <b>37</b>      | <b>8</b>     | <b>0</b>            | <b>8.9%</b>          | <b>6.1</b>        | <b>5</b>    |
| May                 | 19             | 3            | 0                   | 19.6%                | 5.9               | 2           |
| September           | 28             | 6            | 0                   | 1.3%                 | 6.2               | 4           |
| <b>Gorge</b>        | <b>34</b>      | <b>6</b>     | <b>1</b>            | <b>20.5%</b>         | <b>6.1</b>        | <b>3</b>    |
| May                 | 20             | 3            | 1                   | 34.5%                | 6.4               | 1           |
| September           | 27             | 5            | 1                   | 11.7%                | 5.9               | 3           |

## 2018 Results

The metrics for 2018 sample sites show high stream quality, most like 2017 than previous years, and the samples included 64 different taxa across all samples (Table 4). All samples showed exceptionally high taxon richness values, with the Gorge site being at a similar level to the other sites. All sites had at least two EPT taxa present, and again the only Plecoptera specimens found this year were at the Gorge site in both samples. The percent Chironomidae metric was slightly lower across most of the sites compared to previous years. Like 2017, the highest percent Chironomidae value was in the May Headwaters sample, but again the community consisted mostly of moderate-tolerance species. The average tolerance values are like previous years, and all samples had some intolerant taxa present this year, so the sites also provide suitable conditions for these species.

Table 4. Metrics for each sample site in 2018

| Site                | Taxon Richness | EPT Richness | Plecoptera Richness | Percent Chironomidae | Average Tolerance | Intolerance |
|---------------------|----------------|--------------|---------------------|----------------------|-------------------|-------------|
| <b>Headwaters</b>   | <b>35</b>      | <b>6</b>     | <b>0</b>            | <b>18.7%</b>         | <b>5.9</b>        | <b>4</b>    |
| May                 | 24             | 2            | 0                   | 35.8%                | 5.7               | 2           |
| September           | 26             | 5            | 0                   | 4.9%                 | 6.0               | 3           |
| <b>Middle Reach</b> | <b>37</b>      | <b>6</b>     | <b>0</b>            | <b>7.3%</b>          | <b>6.0</b>        | <b>4</b>    |
| May                 | 21             | 3            | 0                   | 13.8%                | 5.9               | 2           |
| September           | 25             | 4            | 0                   | 0.9%                 | 6.1               | 3           |
| <b>Gorge</b>        | <b>36</b>      | <b>8</b>     | <b>1</b>            | <b>11.5%</b>         | <b>5.7</b>        | <b>5</b>    |
| May                 | 27             | 6            | 1                   | 17.9%                | 5.9               | 3           |
| September           | 24             | 5            | 1                   | 5.4%                 | 5.6               | 3           |

## 2019 Results

In 2019, the samples included 58 unique taxa and showed an ongoing trend of high stream quality (Table 5). The taxon richness values continue to show high levels of diversity throughout Brown's Creek. The May Headwaters community had a richness level higher than any sample in this project so far with over 30 unique taxa. The Middle Reach and Gorge sites showed diversity like previous years. All samples had at least 3 unique EPT taxa present, with the Gorge site showing the only Plecoptera specimens. However, this year both *Isoperla* and *Haploperla* were found at this site, which have moderately low tolerance values of 4.2 and 4.0, respectively.

The Chironomidae proportion was slightly higher in 2019 than in previous years in the Headwaters and Middle Reach sites with half to two-thirds of the May samples comprised of midges. This level of community domination would generally indicate a higher level of impact, although the majority of the Chironomidae community was again represented by *Diamesa*. The average tolerance values are also slightly lower than in previous years with all the samples remaining below 6.0, and all sites included intolerant taxa. This indicates that the stream community is stable and continuing to support the species that are intolerant to stream impacts.

Table 5. Metrics for each sample site in 2019

| Site                | Taxon Richness | EPT Richness | Plecoptera Richness | Percent Chironomidae | Average Tolerance | Intolerance |
|---------------------|----------------|--------------|---------------------|----------------------|-------------------|-------------|
| <b>Headwaters</b>   | <b>39</b>      | <b>8</b>     | <b>0</b>            | <b>40.3%</b>         | <b>5.7</b>        | <b>4</b>    |
| May                 | 32             | 4            | 0                   | 67.0%                | 5.5               | 2           |
| September           | 23             | 6            | 0                   | 16.9%                | 6.0               | 3           |
| <b>Middle Reach</b> | <b>32</b>      | <b>9</b>     | <b>0</b>            | <b>28.4%</b>         | <b>5.6</b>        | <b>5</b>    |
| May                 | 19             | 3            | 0                   | 54.8%                | 5.2               | 3           |
| September           | 20             | 7            | 0                   | 4.0%                 | 5.9               | 4           |
| <b>Gorge</b>        | <b>31</b>      | <b>9</b>     | <b>2</b>            | <b>11.3%</b>         | <b>5.9</b>        | <b>4</b>    |
| May                 | 24             | 6            | 1                   | 25.3%                | 5.9               | 3           |
| September           | 17             | 5            | 1                   | 1.8%                 | 5.9               | 2           |

## 2020 Results

In 2020, the samples included 54 unique taxa and showed an ongoing trend of high stream quality (Table 6). The taxon richness values continue to show high levels of diversity throughout Brown’s Creek. All samples had at least 3 unique EPT taxa present. The Middle Reach and Gorge sites both showed Plecoptera specimens. This is the first year that Plecoptera has been found in the Middle Reach which could represent higher water quality in that area than in years past.

The average tolerance values for the samples were similar to 2019, with numbers falling between the 5.6 – 6.0 range which are slightly lower than in previous years. Each site had some intolerant taxa found which indicates that the stream community is stable and continuing to support the species that are intolerant to stream impacts.

This year there was a higher number of taxa with moderately low tolerance values ranging from 4.1 to 4.5. *Isoperla* (TV=4.2) was found in both Middle Reach and Gorge, *Ptilostomis* (TV=4.4) and *Pycnopsyche* (TV=4.5) were found in Headwaters, and *Antocha* (TV=4.1) was found in Gorge. A higher number of taxa with moderately low tolerance values is another indicator of good water quality. The Chironomidae proportion was lower in 2020 compared to 2019 where we saw the highest numbers of any year sampled. Similar to 2019, the majority of the Chironomidae community was again represented by *Diamesa*, which has a moderate tolerance value compared to other midges.

Table 6. Metrics for each sample site in 2020

| Site                | Taxon Richness | EPT Richness | Plecoptera Richness | Percent Chironomidae | Average Tolerance | Intolerance |
|---------------------|----------------|--------------|---------------------|----------------------|-------------------|-------------|
| <b>Headwaters</b>   | <b>36</b>      | <b>7</b>     | <b>0</b>            | <b>23.8%</b>         | <b>5.8</b>        | <b>2</b>    |
| May                 | 22             | 3            | 0                   | 35.9%                | 5.7               | 2           |
| September           | 25             | 6            | 0                   | 11.4%                | 6.0               | 3           |
| <b>Middle Reach</b> | <b>32</b>      | <b>10</b>    | <b>1</b>            | <b>18.1%</b>         | <b>5.7</b>        | <b>5</b>    |
| May                 | 16             | 6            | 1                   | 28.5%                | 5.6               | 3           |
| September           | 22             | 5            | 0                   | 18.1%                | 5.7               | 3           |
| <b>Gorge</b>        | <b>29</b>      | <b>7</b>     | <b>1</b>            | <b>14.2%</b>         | <b>5.7</b>        | <b>5</b>    |
| May                 | 20             | 5            | 1                   | 21.1%                | 5.7               | 2           |
| September           | 22             | 7            | 1                   | 6.6%                 | 5.7               | 5           |

## 2021 Results

In 2021, the samples included 52 unique taxa and showed an ongoing trend of high stream quality (Table 7). The taxon richness values continue to show high levels of diversity throughout Brown's Creek. All samples had at least 4 unique EPT taxa present. The Gorge site showed Plecoptera specimens for both sample occurrences.

The average tolerance values for the samples in 2021 were similar to 2020, with numbers falling between the 5.6 – 6.0 range which are slightly lower than in previous years. Each site had some intolerant taxa found which indicates that the stream community is stable and continuing to support the species that are intolerant to stream impacts.

This year again there was a higher number of taxa with moderately low tolerance values ranging from 4.1 to 4.5. *Ptilostomis* (TV=4.4) was found in Headwaters. *Isoperla* (TV=4.2), *Pycnopsyche* (TV=4.5) and *Antocha* (TV=4.1) were found in Gorge. A higher number of taxa with moderately low tolerance values is another indicator of good water quality.

The Chironomidae proportion was lower in 2021 compared to 2020 and substantially lower to 2019 where we saw the highest numbers of any year sampled. The majority of the Chironomidae community was represented by *Polypedilum*, which has a high tolerance value compared to other midges. The second highest number of midges were represented by *Diamesa* which has a lower tolerance value and has been the most prevalent genus found in past years. The lower Chironomidae numbers this year are a good sign since most of MN had experienced low water conditions over the summer of 2021. Lower water conditions usually result in warmer water temperatures which helps Chironomidae development. EPT richness remained stable from Spring to fall sampling and increased in the Middle Reach which is similar to past years and a great sign that taxa can maintain richness even in low water conditions.

Table 7. Metrics for each sample site in 2021

| Site                | Taxon Richness | EPT Richness | Plecoptera Richness | Percent Chironomidae | Average Tolerance | Intolerance |
|---------------------|----------------|--------------|---------------------|----------------------|-------------------|-------------|
| <b>Headwaters</b>   | <b>28</b>      | <b>8</b>     | <b>0</b>            | <b>6.0%</b>          | <b>5.5</b>        | <b>5</b>    |
| May                 | 20             | 5            | 0                   | 9.7%                 | 5.3               | 4           |
| September           | 21             | 5            | 0                   | 2.4%                 | 5.8               | 3           |
| <b>Middle Reach</b> | <b>40</b>      | <b>9</b>     | <b>0</b>            | <b>15.6%</b>         | <b>5.7</b>        | <b>5</b>    |
| May                 | 25             | 4            | 0                   | 30.8%                | 6.3               | 3           |
| September           | 24             | 7            | 0                   | 3.1%                 | 5.1               | 3           |
| <b>Gorge</b>        | <b>28</b>      | <b>7</b>     | <b>1</b>            | <b>14.7%</b>         | <b>5.8</b>        | <b>3</b>    |
| May                 | 22             | 5            | 1                   | 24.9%                | 6.1               | 2           |
| September           | 16             | 5            | 1                   | 4.5%                 | 5.5               | 2           |

## 2022 Results

In 2022, the samples included 50 unique taxa and showed an ongoing trend of high stream quality (Table 8). The taxon richness values continue to show high levels of diversity throughout Brown's Creek. All samples had at least 5 unique EPT taxa present. All samples had at least 20 unique taxa with the highest being 28 representing a healthy and diverse community of invertebrates. The Gorge site showed Plecoptera specimens for both sample occurrences. Middle Reach showed plecoptera specimens for its spring sample.

The average tolerance values were like years past for Headwaters and Middle Reach. Gorge site had a normal average tolerance in the spring; but showed a much lower average tolerance value for its fall sample due to the abundance of the caddisfly *Glossosoma* (TV=1.1), *Protoptila* (TV=1.4) and riffle beetle *Optioservus* (TV=3.1). Each site had at least 5 intolerant taxa which indicates that the stream community is stable and continuing to support the species that are intolerant to stream impacts. The Chironomidae proportion was average for Headwaters which typically shows higher numbers than the other two sites. The majority of the Chironomidae community was represented by *Diamesa* (TV=5.0), which has a lower tolerance value and has been the most prevalent genus found in past years. Middle Reach and Gorge came back with lower-than-average Chironomidae numbers which are similar to 2015 where we had the lowest numbers of all year's sampled.

Table 8. Metrics for each sample site in 2022

| Site                | Taxon Richness | EPT Richness | Plecoptera Richness | Percent Chironomidae | Average Tolerance | Intolerance |
|---------------------|----------------|--------------|---------------------|----------------------|-------------------|-------------|
| <b>Headwaters</b>   | <b>32</b>      | <b>7</b>     | <b>0</b>            | <b>26.2%</b>         | <b>6.0</b>        | <b>8</b>    |
| May                 | 25             | 5            | 0                   | 41.4%                | 5.3               | 6           |
| September           | 22             | 5            | 0                   | 10.8%                | 6.3               | 6           |
| <b>Middle Reach</b> | <b>37</b>      | <b>10</b>    | <b>1</b>            | <b>4.5%</b>          | <b>5.6</b>        | <b>7</b>    |
| May                 | 25             | 8            | 1                   | 5.1%                 | 5.3               | 5           |
| September           | 28             | 7            | 0                   | 4.0%                 | 5.8               | 6           |
| <b>Gorge</b>        | <b>33</b>      | <b>10</b>    | <b>1</b>            | <b>3.9%</b>          | <b>5.1</b>        | <b>8</b>    |
| May                 | 26             | 7            | 1                   | 3.5%                 | 6.1               | 5           |
| September           | 20             | 8            | 1                   | 4.2%                 | 4.2               | 7           |

## 2023 Results

In 2023, the samples included 44 unique taxa and showed an ongoing trend of high stream quality (Table 9). The taxon richness values continue to show high levels of diversity throughout Brown's Creek. All samples had at least 5 unique EPT taxa present. All samples had at least 20 unique taxa with the highest being 26 representing a healthy and diverse community of invertebrates. The Gorge and Middle Reach sites showed Plecoptera specimens for each sample occurrence.

The average tolerance values were like years past for all three sites. Each site had at least 3 intolerant taxa which is consistent with past years indicating that the stream community is stable and continuing to support the species that are intolerant to stream impacts.

The Chironomidae proportion was average for Headwaters which typically shows higher numbers than the other two sites. The majority of the Chironomidae community was represented by *Tvetenia Bavarica* Group (TV=5.0), which has a lower tolerance value than other midges. Middle Reach and Gorge came back with lower-than-average Chironomidae numbers.

Table 9. Metrics for each sample site in 2023

| Site                | Taxon Richness | EPT Richness | Plecoptera Richness | Percent Chironomidae | Average Tolerance | Intolerance |
|---------------------|----------------|--------------|---------------------|----------------------|-------------------|-------------|
| <b>Headwaters</b>   |                |              |                     |                      |                   |             |
| September           | 20             | 5            | 0                   | 10.3%                | 5.7               | 3           |
| <b>Middle Reach</b> |                |              |                     |                      |                   |             |
| September           | 26             | 7            | 1                   | 4.0%                 | 5.6               | 3           |
| <b>Gorge</b>        |                |              |                     |                      |                   |             |
| September           | 21             | 8            | 1                   | 5.4%                 | 5.1               | 4           |

## 2024 Results

In 2024, the samples included 38 unique taxa and showed an ongoing trend of high stream quality (Table 10). The taxon richness values continue to show high levels of diversity throughout Brown's Creek. All samples had at least 5 unique EPT taxa present. All samples had at least 21 unique taxa with the highest being 25 representing a healthy and diverse community of invertebrates. The Gorge and Middle Reach sites showed Plecoptera specimens for each sample occurrence.

The average tolerance values were like years past for all three sites. Each site had at least 3 intolerant taxa which is consistent with past years indicating that the stream community is stable and continuing to support the species that are intolerant to stream impacts.

The Chironomidae proportion was lower for Headwaters which typically shows higher numbers than the other two sites. Middle Reach and Gorge came back with average Chironomidae numbers.

Table 10. Metrics for each sample site in 2024

| Site                | Taxon Richness | EPT Richness | Plecoptera Richness | Percent Chironomidae | Average Tolerance | Intolerance |
|---------------------|----------------|--------------|---------------------|----------------------|-------------------|-------------|
| <b>Headwaters</b>   |                |              |                     |                      |                   |             |
| September           | 24             | 7            | 0                   | 6.3%                 | 5.8               | 4           |
| <b>Middle Reach</b> |                |              |                     |                      |                   |             |
| September           | 25             | 5            | 1                   | 7.8%                 | 5.9               | 2           |
| <b>Gorge</b>        |                |              |                     |                      |                   |             |
| September           | 21             | 6            | 1                   | 5.2%                 | 5.4               | 3           |

## 2025 Results

In 2025, the samples included 30 unique taxa and showed an ongoing trend of high stream quality (Table 11). The taxon richness values continue to show high levels of diversity throughout Brown's Creek. All samples had at least 1 unique EPT taxa present. EPT numbers were lower than in past years which could be caused by low water conditions. All samples had at least 11 unique taxa with the highest being 21 representing a healthy and diverse community of invertebrates. The Gorge site showed 1 Plecoptera specimen.

The average tolerance values were like years past for all three sites. Each site had at least 2 intolerant taxa which is consistent with past years indicating that the stream community is stable and continuing to support the species that are intolerant to stream impacts.

The Chironomidae proportion showed low numbers which is a good indicator. This taxa group tends to dominate in heavily impacted streams, so this low proportion of the community means that there are likely minimal high-impact pollutant sources affecting the stream.

Table 11. Metrics for each sample site in 2025

| Site                | Taxon Richness | EPT Richness | Plecoptera Richness | Percent Chironomidae | Average Tolerance | Intolerance |
|---------------------|----------------|--------------|---------------------|----------------------|-------------------|-------------|
| <b>Headwaters</b>   |                |              |                     |                      |                   |             |
| September           | 21             | 4            | 0                   | 2.7%                 | 6.0               | 3           |
| <b>Middle Reach</b> |                |              |                     |                      |                   |             |
| September           | 16             | 1            | 0                   | 1.6%                 | 6.3               | 2           |
| <b>Gorge</b>        |                |              |                     |                      |                   |             |
| September           | 11             | 4            | 1                   | 1.9%                 | 5.7               | 2           |

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## 2016 – 2025 Comparisons

General macroinvertebrate metrics are best used in combination to determine the health of a stream ecosystem. However, a few of the metrics can give an overall glimpse into how stream health is changing over time. The taxa richness represents how many unique specimens are present in a sample, which is an indication of biological community stability. Streams with high taxa richness are better able to respond to and recover from impacts to the water quality. In this project, the taxa richness for all samples ranges from 11 to 32, and over the years of this project, the communities present appear to be stable and show an increasing trend in richness (Figure 3). This indicates that the stream ecosystem is healthy and successfully recovering from any disturbances or impacts that may have occurred in the years prior to the survey.

The Headwaters sample from May 2019 showed 32 unique taxa, which was higher than in any of the previous samples, indicating that the stream has a very stable and diverse community present. Samples from 2021 showed lower taxa richness than 2019 but remained comparable with earlier years of sampling. 2022 showed continued improvement. We had a record low for average tolerance from the fall Gorge site which also resulted in a record high in IBI scoring from that same site. The total tolerance comparison across all years shows a strong increase in intolerant taxa along with a decrease in tolerant taxa. Decreasing tolerance values along with stable taxa richness and lower Chironomidae numbers are all great indicators that conditions are improving for Brown's Creek. When comparing 2023's results with past numbers for taxa richness, average tolerance and IBI scoring; consistent stable numbers appear to be the trend which is a great sign that no new impairments have been introduced to Brown's Creek and the stream seems to be maintaining a healthy macroinvertebrate community. 2024's results remained consistent regarding taxa richness, EPT species and percent Chironomidae and average tolerance. MIBI numbers were great this year with both Middle Reach and Gorge coming in above the exceptional use threshold. Head waters had a lower MIBI score but still came in consistent with past years. 2025's results showed a lower taxa richness and EPT species than last year but were still comparable to years past. MIBI numbers were consistent with past years with numbers well above the general use threshold.

The average tolerance value metrics can also give a good insight into stream health since it consists of a weighted average calculation. The Brown's Creek samples show an interesting pattern over the course of the years surveyed (Figure 4). Natural fluctuations in community composition can occur year to year and are a normal occurrence in this tolerance range of 5.5 to 6.4. There is a slight decreasing trend developing over the years, showing that the stream community can support more specimens that are intolerant to impacts. This is an indication of good water quality and a stable aquatic ecosystem. The Headwaters and Middle Reach sites tend to follow the same pattern throughout the sample period, indicating similar conditions at those two sites. However, the Gorge samples follow a different pattern, showing a higher tolerance score in 2016 and 2017 when the

other two sites showed much lower scores. This could be due to a disturbance or impact occurring to Brown's Creek between the Middle Reach and Gorge sample sites. However, the disturbance is not severe enough to have strongly altered the other metrics in the Gorge samples, so the stream community is able to recover before reaching this last sample site. In 2020 & 2021, we see more consistent taxa in Headwaters and Middle Reach. Gorge showed more variation than in years past when compared with the other two sites; this could be due to low water levels in the fall. There is a down trend in average tolerance values and it seems that the numbers of intolerant taxa have been rising year after year which is a great sign that conditions are improving. Even though numbers of unique taxa have declined since 2018 in Brown's creek, the taxa with lower tolerance values have been increasing. As conditions improve in Brown's creek it allows taxa with lower tolerance values a chance to rebound and increase their populations.

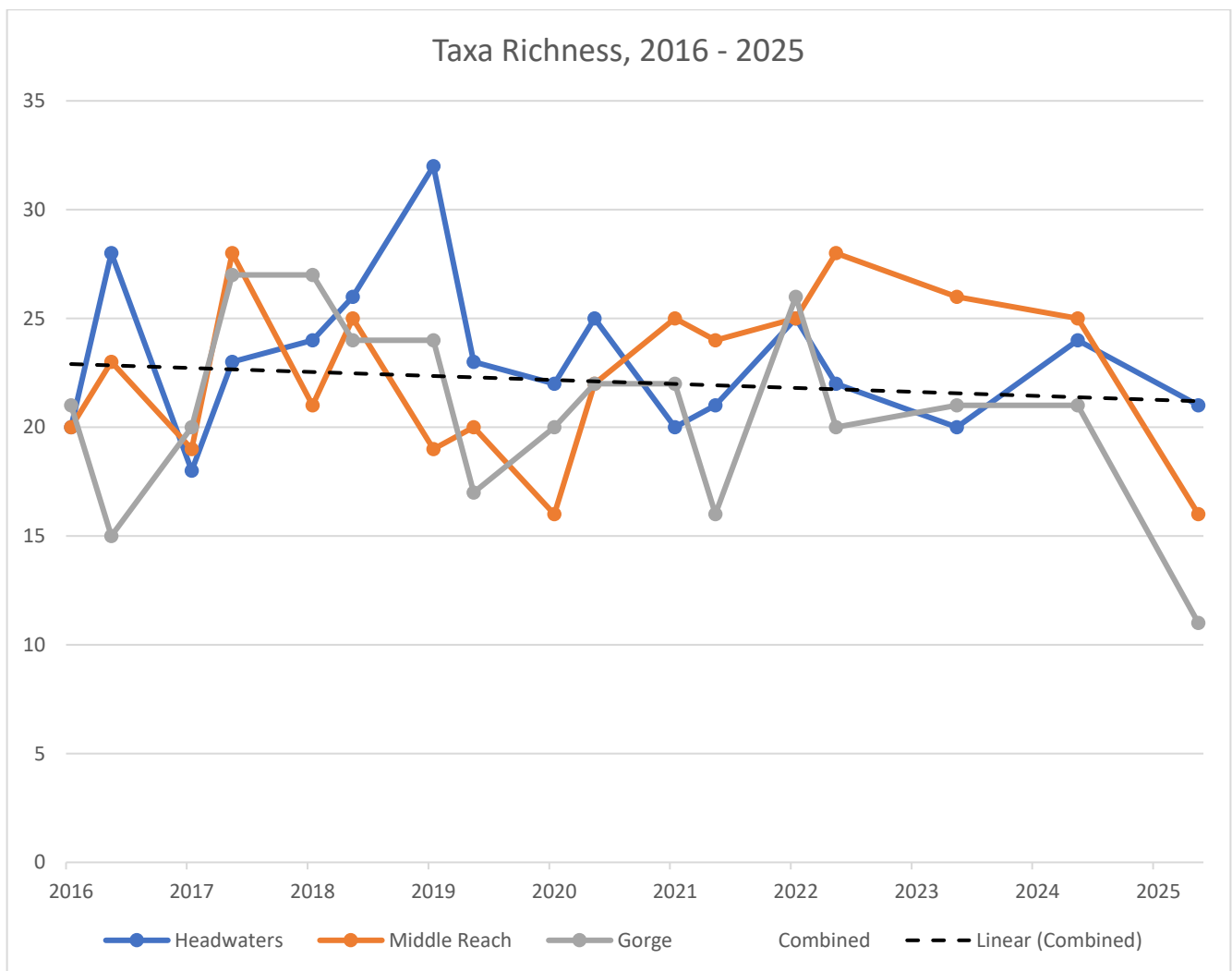


Figure 3: Taxa richness values for Brown's Creek samples from 2016 to 2025

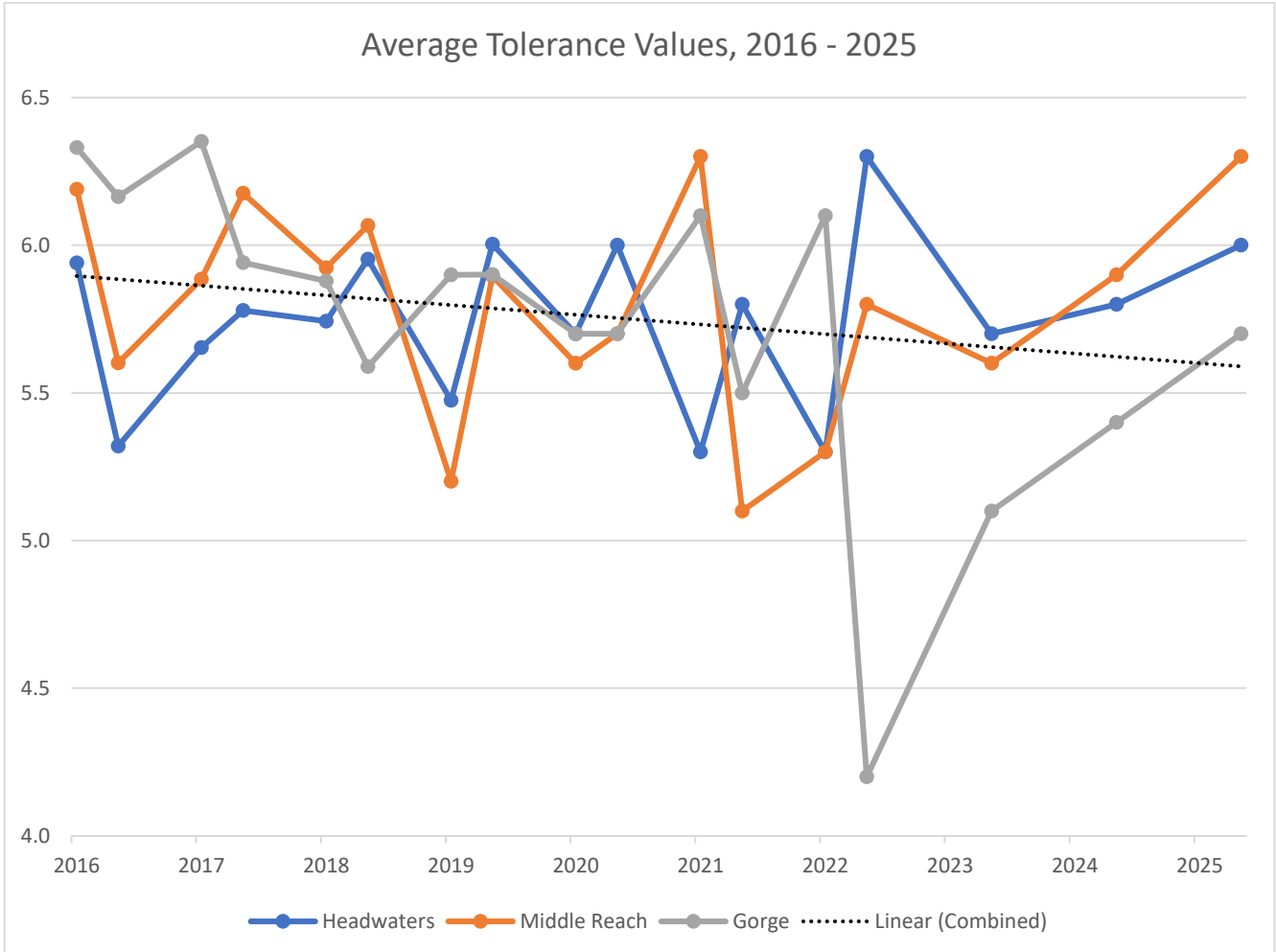


Figure 4: Average tolerance values for Brown's Creek samples from 2016 to 2025

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## *Index of Biological Integrity (IBI)*

The MPCA has developed a state-wide method of evaluating stream health using aquatic macroinvertebrates. This index gives each sample a numerical value that can be used to compare one site to another. It can also be used to monitor individual sites over time to determine whether the stream condition is improving or declining.

Due to the geographic differences throughout Minnesota and the variability in stream types, the state has been divided up into three regions that comprise nine different invertebrate stream classes (Figure 2). Each class has a different IBI calculation that best represents the invertebrate communities typically found within the region. They are based primarily on region, watershed size, thermal regime, and stream gradient (MPCA). The study area in this project is located within the Southern Coldwater Streams invertebrate class.

## *Tiered Aquatic Life Uses (TALU)*

Stream health throughout Minnesota is evaluated for its capacity to sustain aquatic life, including the macroinvertebrates, fish, plants, and other organisms. The MPCA developed models with threshold IBI values that represent how well the stream can sustain aquatic life. These include *Exceptional Use* for high-quality streams, *General Use* for streams with light impacts, and *Modified Use* for areas with heavy impacts to the streams (Table 7). Each invertebrate stream class has different threshold levels based on the invertebrate communities typically found in that region. In this project, almost all samples were above the General Use Threshold, and several were above the Exceptional Use Threshold.

**Table 12: Tiered Aquatic Life Uses as determined by the MPCA (MPCA 2014)**

| <b>Use Category</b>    | <b>Description</b>   |
|------------------------|--|
| <b>Exceptional Use</b> | <b>Evident changes in structure due to loss of some rare native taxa; shifts in relative abundance; ecosystem level functions fully maintained</b>                     |
| <b>General Use</b>     | <b>Overall balanced distribution of all expected major groups; ecosystem functions largely maintained through redundant attributes</b>                                 |
| <b>Modified Use</b>    | <b>Sensitive taxa markedly diminished; conspicuously unbalanced distribution of major taxonomic groups; ecosystem function shows reduced complexity and redundancy</b> |

The Southern Coldwater Streams region represents areas in the southern portions of Minnesota with deciduous broadleaf forests. This invertebrate class has an IBI General Use Threshold of 43 and an Exceptional Use Threshold of 72. In this project, almost all samples met the General Use Threshold, and several of the Middle Reach & Gorge samples exceeded the Exceptional Use Threshold as well as all the sites in September of 2019 (Table 7, Figure 5). The highest score was 86.5 from the September 2022 sample of the Gorge site and the lowest was 35.2 from the May 2017 sample of the Gorge site. There are natural fluctuations in the invertebrate community, causing the IBI scores to change over time. The samples taken in the fall of each year usually show a higher score than the spring samples, but overall, the scores are between the General and Exceptional Use Thresholds, indicating a stable aquatic community.

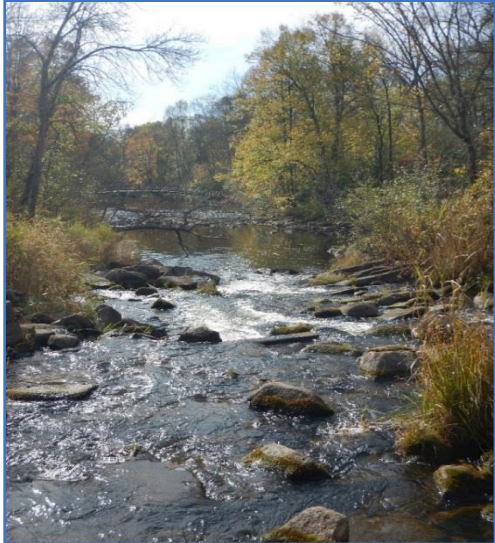


Figure 5: Example of a Southern Coldwater Stream sample site

| Sample Date |           | Headwaters | Middle Reach | Gorge |
|-------------|-----------|------------|--------------|-------|
| 2016        | May       | 51.7       | 44.8         | 41    |
|             | September | 63         | 77.7         | 65.8  |
| 2017        | May       | 49.8       | 56.1         | 35.2  |
|             | September | 65.1       | 81.1         | 61.4  |
| 2018        | May       | 53.8       | 66.6         | 52.4  |
|             | September | 61.2       | 68.4         | 58.9  |
| 2019        | May       | 49.9       | 48.9         | 51    |
|             | September | 73.1       | 86.4         | 82    |
| 2020        | May       | 63.3       | 64.5         | 53.2  |
|             | September | 57.6       | 76.6         | 86.2  |
| 2021        | May       | 72.5       | 43.3         | 48.4  |
|             | September | 77.8       | 68.9         | 82    |
| 2022        | May       | 59.1       | 75.1         | 78    |
|             | September | 52.7       | 78.5         | 86.5  |
| 2023        | September | 59.5       | 72.7         | 77.8  |
| 2024        | September | 73.1       | 61.1         | 89.9  |
| 2025        | September | 60.88      | 58.68        | 78.7  |

Table 12: Index of Biological Integrity (IBI) scores for Brown’s Creek samples from 2016 to 2025

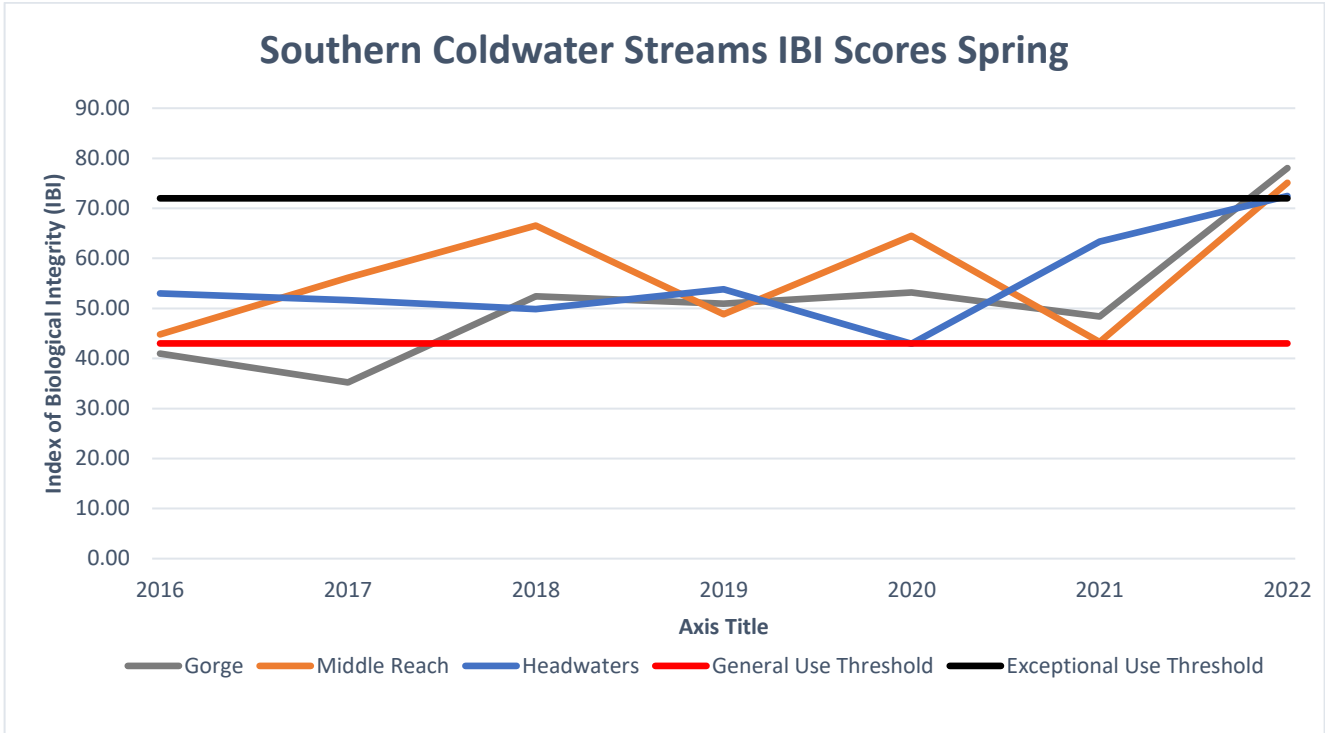


Figure 6: IBI scores, General Use Threshold, and Exceptional Use Threshold for Brown's Creek samples within the Southern Coldwater Streams class in spring 2016 – 2022

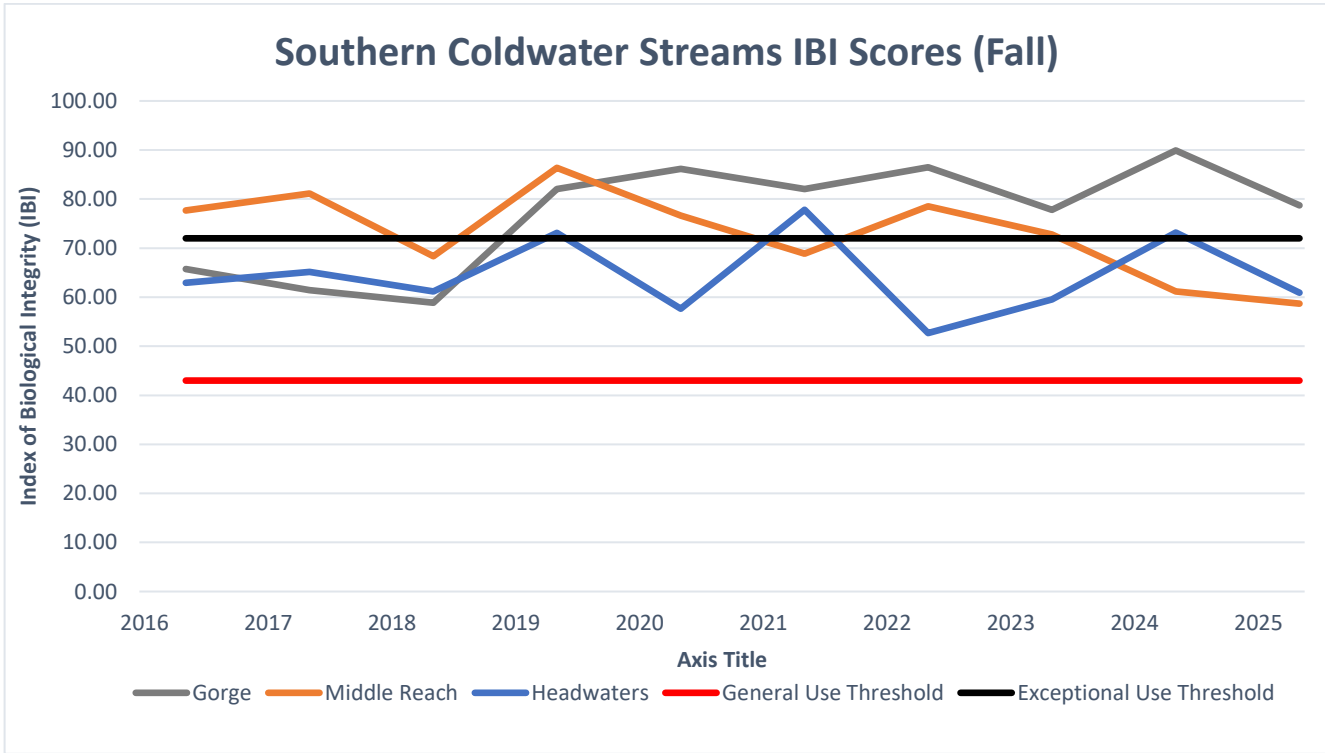


Figure 7: IBI scores, General Use Threshold, and Exceptional Use Threshold for Brown's Creek samples within the Southern Coldwater Streams class in fall 2016 – 2025

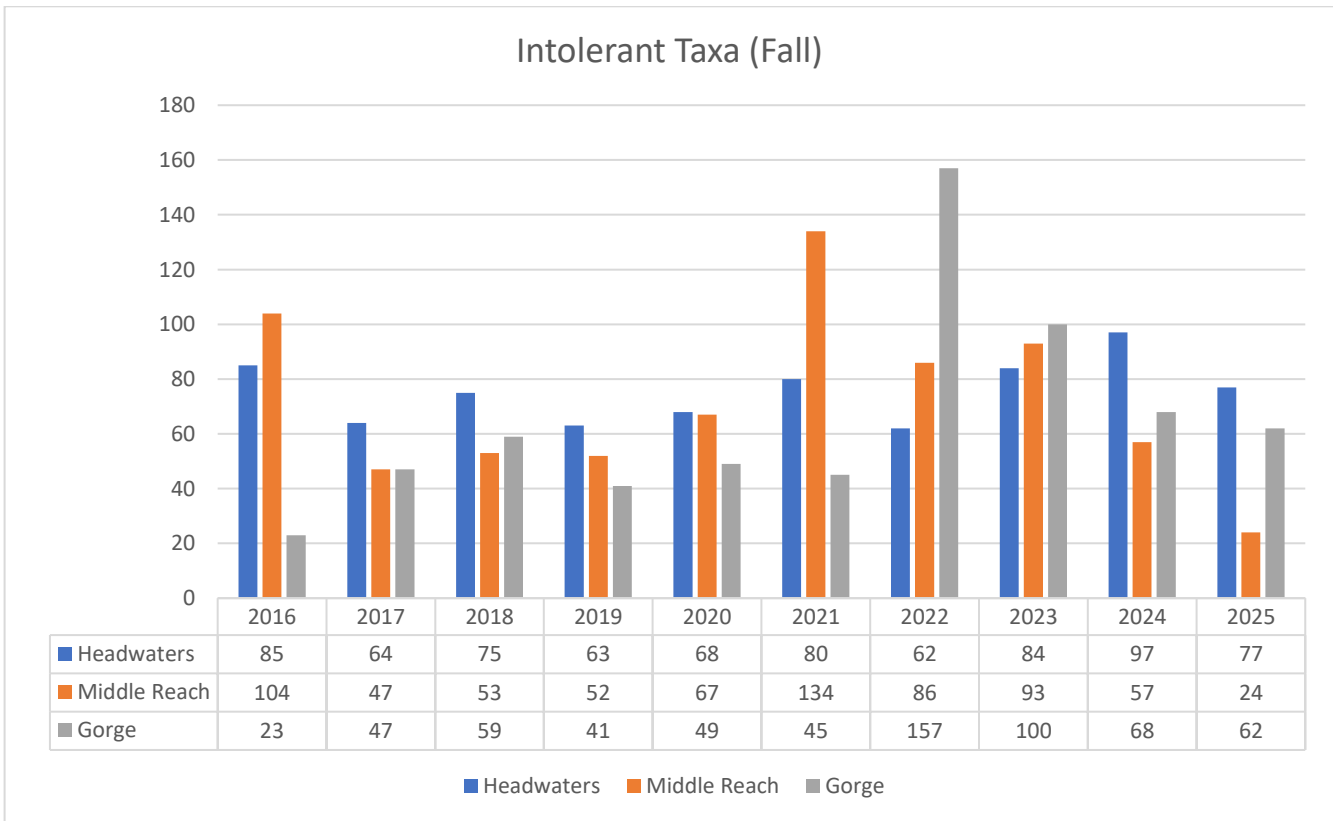
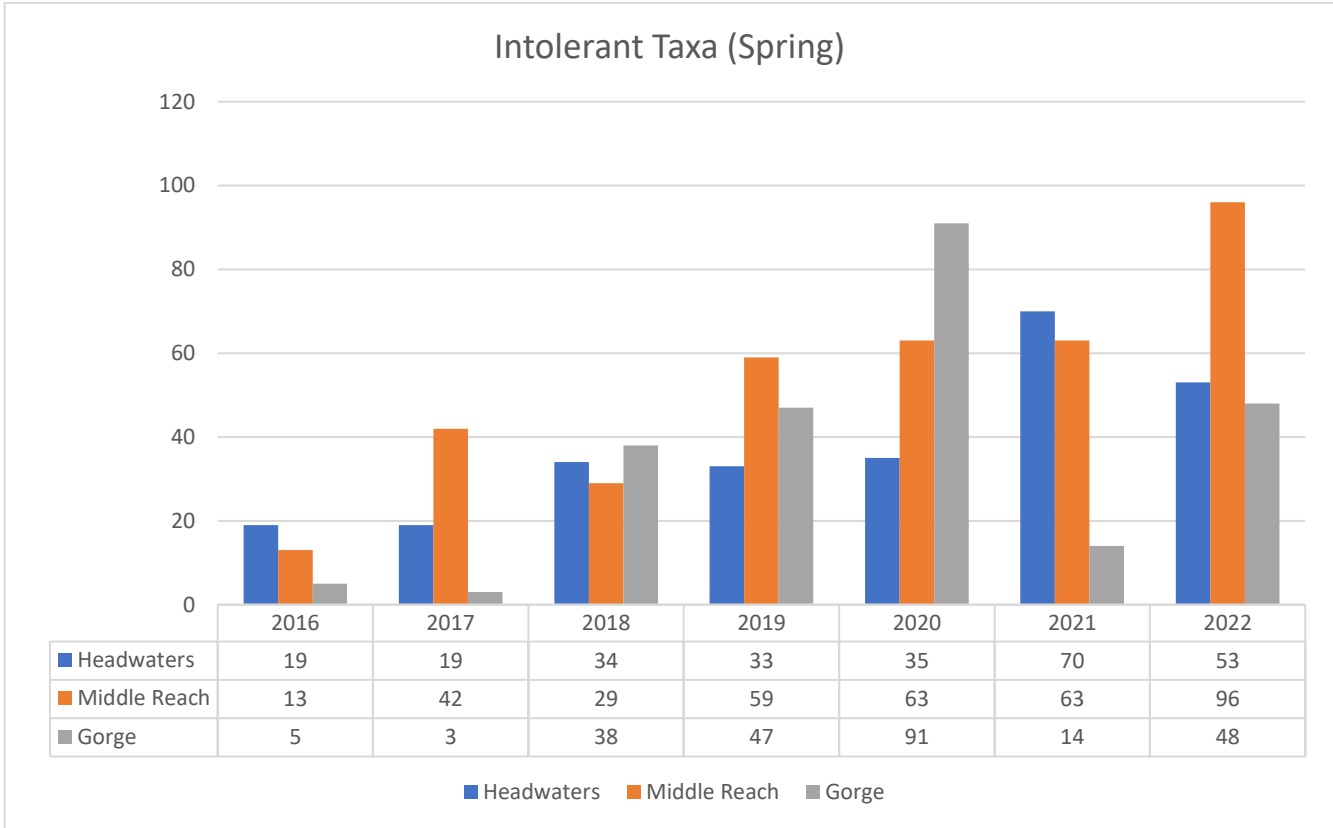


Figure 8 & 9: Comparison of Intolerant taxa spring vs fall for Brown's Creek 2016 - 2025

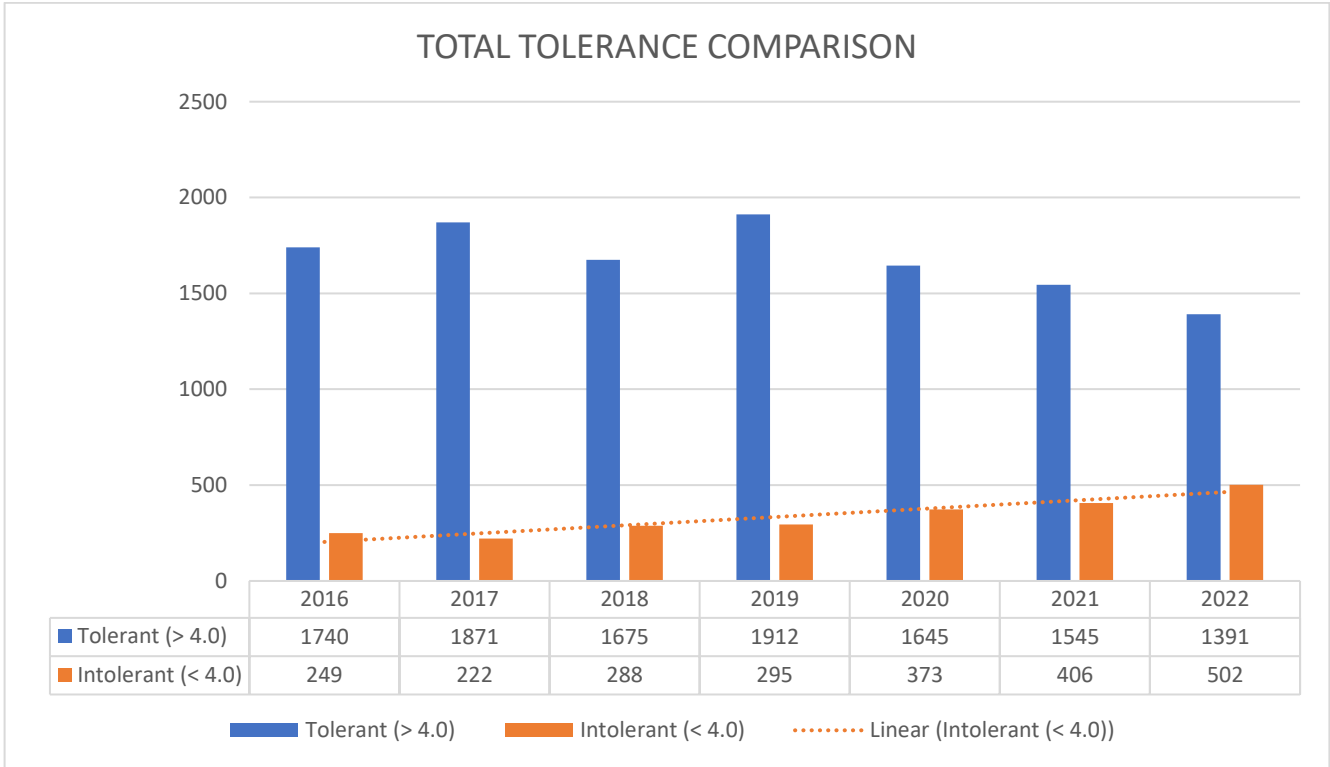


Figure 10: Comparison of Tolerant from Intolerant Taxa for Brown's Creek 2016 – 2022

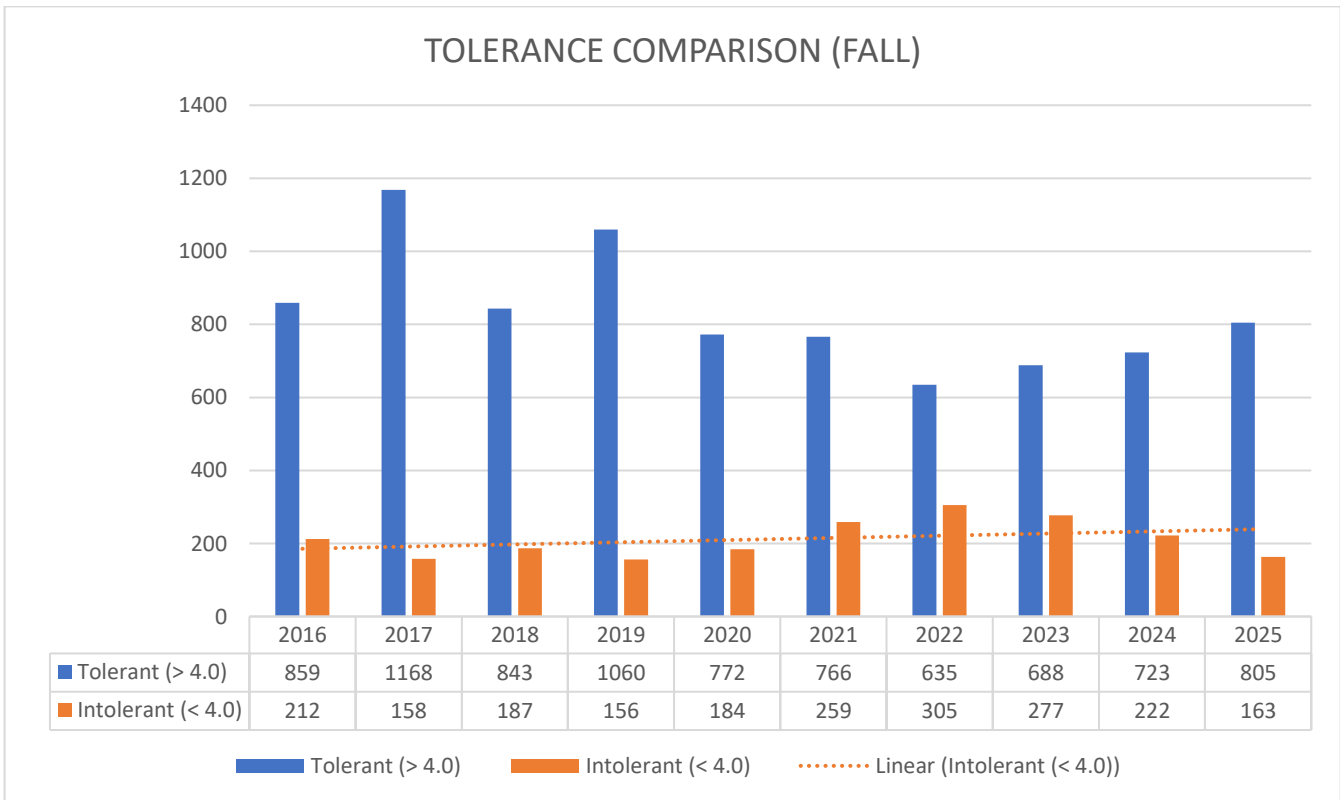


Figure 11: Comparison of Tolerant from Intolerant Taxa for Brown's Creek (Fall) 2016 – 2025

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## Discussion

### *General Metrics*

The macroinvertebrate communities sampled throughout this project included a wide variety of species, and the sample sites showed a range of metric results. Overall, there were 161 unique taxa across all the years of sampling, meaning that the Brown's Creek sites have diverse communities with seasonal fluctuations in the community composition (Appendix 1). The most prevalent taxa overall were scuds (*Gammarus*), blackflies (*Simulium*), and mayflies (*Baetis*). These taxa have medium-level tolerance values, so they are often found in higher densities in streams with moderate impacts. The dominance of tolerant taxa like these can cause the average tolerance value of a sample to be high. Most of the samples in this project had an average tolerance value between 5 and 7, with the lowest being 5.2 in the May 2019 Middle Reach sample and the highest being 6.4 in the May 2017 Gorge sample.

Despite the prevalence of tolerant species, all the samples included some intolerant taxa, indicating that the level of impacts on the streams was not high enough to prevent the sensitive species from living there. The next most abundant taxon was a riffle beetle (*Optioservus*) which is intolerant to impacts with a tolerance value of only 3.1. The abundance of these riffle beetles indicates that the stream is clean and fast enough to support a strong community of intolerant taxa. Intolerant taxa are any species with a tolerance value (TV) of 4 or less. In this project, these included *Cardiocladius* (TV = 2.7), *Glossosoma* (TV = 1.1), *Lepidostoma* (TV = 0.1), *Limnephilidae* (TV = 3.5), *Lype* (TV = 3.1), *Neophylax* (TV = 3.2), *Optioservus* (TV = 3.1), *Parapsyche* (TV = 1.0), *Perlodidae* (TV = 2.7), *Prosimulium* (TV = 3.0), *Protoptila* (TV = 1.4), and *Rhyacophila* (TV = 0.0). This combination of taxa shows that while these streams likely have some urbanization impact, they also have pockets of good microhabitat and sufficient oxygen.

The EPT metric evaluates the diversity of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) in the samples. These insect groups are generally indicators of less impacted waters since they contain many intolerant species. In this project, the EPT values ranged from 1 to 7 unique taxa in each sample. While there are no definitive thresholds with this metric, sites with few or no EPT taxa likely have a substantial number of impacts and may be targeted for management practices to improve the watersheds that flow into these sites. The Plecoptera subset of the EPT metric is also evaluated since the stonefly group contains mostly intolerant species, and typically they require high-quality, well-oxygenated water. Two unique stonefly species were found during this project (*Isoperla* and *Haploperla*), and they have only been found in the Gorge & Middle Reach Sites. This group of insects is typically not very diverse in stream samples without strong riffles present to keep the water full of dissolved oxygen.

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The Chironomidae fraction of a sample can also indicate general water quality. Even though this group is very diverse and includes midge species with tolerance values ranging from 0 to 10, generally they only dominate a sample at a site with heavy pollution impacts. The samples in this project ranged from 0% to 67% Chironomidae present, with some of the largest proportions seen in the May 2019 samples. Since most healthy streams have a diverse community of macroinvertebrates, the high numbers of midges seen in the spring samples initially seems like an indication of impact. However, with the change in community throughout the year and with such low Chironomidae proportions in the fall samples, Brown's Creek likely has minimal pollution impacts affecting the water quality, especially when looking at all the metrics in combination.

### *Invertebrate Stream Classes*

Minnesota is divided up into invertebrate stream classes based on three geographic regions so the IBI values can be compared to streams within similar regions. These regions include Northern Forest Streams, Southern Streams, and Prairie Streams. The regions are then further divided based on whether the sample was taken from a site with riffle habitats present or only with glides and pools. This survey was located within the Southern Coldwater Streams class. Samples were taken from 3 dominant habitat types in a given reach per season (Either from riffles, pools, runs, glides, undercut banks, leaf packs, or wood debris.)

Each stream class has unique threshold values indicating the level of support for biological communities living there. The highest tier is the Exceptional Use Threshold which represents the highest quality streams that are providing maximum support for aquatic organisms. The next level is the General Use Threshold, which is the target level for streams that are healthy and functioning despite any impacts to them. The lowest level is the Modified Use Threshold, which represents streams with heavy impacts that may be struggling to adequately support the aquatic communities living in them. Sites with IBI scores at or below the Modified Threshold should be prioritized over others for management practices or restorations to improve the stream health.

The Brown's Creek sites within the Southern Coldwater Streams region have been regularly fluctuating with the seasonal sampling over the years of this project. Most IBI scores fall between the General and Exceptional Use Thresholds. The pattern across the three sites shows increased scores in the fall with numbers closer to the Exceptional Use Threshold. The Spring samples show slightly lower scoring with numbers closer to the general use threshold. This pattern is normal for most streams with fall samples showing a better display of a stream's true macro community. With improving IBI scoring occurring over the summer, it's unlikely that any major pollution impacts are occurring along Brown's Creek between the sample points.

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## *Future Projects*

Further monitoring of these sites is recommended to continue establishing the baseline data for these aquatic communities. Each site is dynamic and seasonally changing, so continuing to collect data helps to eliminate the differences due to natural fluctuations in invertebrate communities. Additionally, if there are suspected pollution inputs to a stream or restoration projects in progress, monitoring before and after these impacts is recommended to assess how the biological community is affected.

## Literature Cited

MPCA. 2014. Development of a macroinvertebrate-based Index of Biological Integrity for assessment of Minnesota's rivers and streams. Minnesota Pollution Control Agency, Environmental Analysis and Outcomes Division, St. Paul, MN.

MPCA. 2014. Development of biological criteria for tiered aquatic life uses: Fish and macroinvertebrate thresholds for attainment of aquatic life use goals in Minnesota streams and rivers. Minnesota Pollution Control Agency, Environmental Analysis and Outcomes Division, St. Paul, MN.

## Appendix 1: Project Taxa List

| <u>Order</u>   | <u>Family</u>   | <u>Genus</u>     | <u>Species</u> |
|----------------|-----------------|------------------|----------------|
| Amphipoda      | Crangonyctidae  | Crangonyx        |                |
| Amphipoda      | Gammaridae      | Gammarus         | lacustrus      |
| Amphipoda      | Hyaellidae      | Hyaella          |                |
| Basommatophora | Physidae        | Physella         |                |
| Basommatophora | Planorbidae     | Micromenetus     |                |
| Bivalvia       | Pisidiidae      | Pisidium         |                |
| Bivalvia       | Pisidiidae      | Sphaerium        |                |
| Bivalvia       | Pisidiidae      | Musculium        |                |
| Cambaridae     |                 |                  |                |
| Clitellata     |                 |                  |                |
| Coleoptera     | Dytiscidae      | Agabus           |                |
| Coleoptera     | Dytiscidae      | Ilybius          |                |
| Coleoptera     | Dytiscidae      | Liodesus         |                |
| Coleoptera     | Dytiscidae      | Uvarus           |                |
| Coleoptera     | Elmidae         | Macronychus      |                |
| Coleoptera     | Elmidae         | Optioservus      |                |
| Coleoptera     | Elmidae         | Stenelmis        |                |
| Coleoptera     | Gyrinidae       | Gyrinus          |                |
| Coleoptera     | Haliplidae      | Peltodytes       |                |
| Coleoptera     | Hydraenidae     | Hydraena         |                |
| Coleoptera     | Hydrophilidae   | Enochrus         |                |
| Coleoptera     | Hydrophilidae   | Hydrobius        |                |
| Coleoptera     | Hydrophilidae   | Hydrochara       |                |
| Coleoptera     | Hydrophilidae   | Hydrochus        |                |
| Coleoptera     | Hydrophilidae   | Tropisternus     |                |
| Coleoptera     | Hydrophilidae   | Berosus          |                |
| Coleoptera     | Scirtidae       | Scirtes          |                |
| Collembola     |                 |                  |                |
| Diptera        | Ceratopogoninae | Bezzia/Palpomyia |                |
| Diptera        | Ceratopogonidae | Ceratopogon      |                |
| Diptera        | Ceratopogonidae | Dasyhelea        |                |
| Diptera        | Ceratopogonidae | Mallochohelea    |                |
| Diptera        | Chironomidae    | Brillia          |                |
| Diptera        | Chironomidae    | Cardiocladius    |                |
| Diptera        | Chironomidae    | Chaetocladius    |                |
| Diptera        | Chironomidae    | Cladotanytarsus  |                |
| Diptera        | Chironomidae    | Conchapelopia    |                |
| Diptera        | Chironomidae    | Corynoneura      |                |
| Diptera        | Chironomidae    | Cricotopus       |                |

|         |              |                                 |             |
|---------|--------------|---------------------------------|-------------|
| Diptera | Chironomidae | Cryptochironomus                |             |
| Diptera | Chironomidae | Diamesa                         |             |
| Diptera | Chironomidae | Diplocladius                    |             |
| Diptera | Chironomidae | Eukiefferiella claripennis gr.  |             |
| Diptera | Chironomidae | Eukiefferiella devonica gr.     |             |
| Diptera | Chironomidae | Eukiefferiella tirolensis group |             |
| Diptera | Chironomidae | Limnophyes                      |             |
| Diptera | Chironomidae | Meropelopia                     |             |
| Diptera | Chironomidae | Micropsectra                    |             |
| Diptera | Chironomidae | Microtendipes                   |             |
| Diptera | Chironomidae | Microtendipes pedellus group    |             |
| Diptera | Chironomidae | Nanocladius                     |             |
| Diptera | Chironomidae | Orthocladius (Orthocladius)     |             |
| Diptera | Chironomidae | Orthocladius (Symposiocladius)  | lignicola   |
| Diptera | Chironomidae | Paracricotopus                  |             |
| Diptera | Chironomidae | Parametriocnemus                |             |
| Diptera | Chironomidae | Paratanytarsus                  | longistilus |
| Diptera | Chironomidae | Paratendipes                    |             |
| Diptera | Chironomidae | Polypedilum                     |             |
| Diptera | Chironomidae | Prodiamesa                      |             |
| Diptera | Chironomidae | Rheocricotopus                  |             |
| Diptera | Chironomidae | Rheotanytarsus                  |             |
| Diptera | Chironomidae | Saetheria                       |             |
| Diptera | Chironomidae | Stenochironomus                 |             |
| Diptera | Chironomidae | Stictochironomus                |             |
| Diptera | Chironomidae | Tanytarsus                      |             |
| Diptera | Chironomidae | Thienemanniella                 |             |
| Diptera | Chironomidae | Thienemannimyia complex         |             |
| Diptera | Chironomidae | Thienemannimyia Gr.             |             |
| Diptera | Chironomidae | Tvetenia                        |             |
| Diptera | Chironomidae | Tvetenia bavarica gr.           |             |
| Diptera | Chironomidae | Zavrelimyia                     |             |
| Diptera | Dixidae      | Dixa                            |             |
| Diptera | Empididae    | Chelifera                       |             |
| Diptera | Empididae    | Hemerodromia                    |             |
| Diptera | Empididae    | Metachela                       |             |
| Diptera | Empididae    | Neoplasta                       |             |
| Diptera | Ephydriidae  |                                 |             |
| Diptera | Limoniidae   | Antocha                         |             |
| Diptera | Limoniidae   | Helius                          |             |
| Diptera | Limoniidae   | Limona                          |             |
| Diptera | Limoniidae   | Molophilus                      |             |

|               |                 |               |             |
|---------------|-----------------|---------------|-------------|
| Diptera       | Pediciidae      | Dicranota     |             |
| Diptera       | Simuliidae      | Simulium      |             |
| Diptera       | Simuliidae      | Prosimulium   |             |
| Diptera       | Simuliidae      | Simulium      |             |
| Diptera       | Stratiomyidae   | Stratiomys    |             |
| Diptera       | Stratiomyidae   | Odontomyia    |             |
| Diptera       | Syrphidae       | Chrysogaster  |             |
| Diptera       | Tipulidae       | Antocha       |             |
| Diptera       | Tabanidae       | Chrysops      |             |
| Diptera       | Tipulidae       | Dicranota     |             |
| Diptera       | Tipulidae       | Hexatoma      |             |
| Diptera       | Tipulidae       | Limnophila    |             |
| Diptera       | Tipulidae       | Limonia       |             |
| Diptera       | Tipulidae       | Ormosia       |             |
| Diptera       | Tipulidae       | Pedicia       |             |
| Diptera       | Tipulidae       | Pilaria       |             |
| Diptera       | Tipulidae       | Tipula        |             |
| Ephemeroptera | Baetidae        | Acentrella    |             |
| Ephemeroptera | Baetidae        | Baetis        |             |
| Gastropoda    | Ancylidae       | Ferrissia     |             |
| Gastropoda    | Lymnaeidae      | Stagnicola    |             |
| Gastropoda    | Physidae        | Aplexa        |             |
| Gastropoda    | Physidae        | Physa         | gyrina      |
| Gastropoda    | Physidae        | Physa         | acuta       |
| Gastropoda    | Physidae        | Physa         | gyrina      |
| Gastropoda    | Planorbidae     | Gyraulus      | parvus      |
| Gastropoda    | Valvatidae      | Valvata       | perdepressa |
| Hemiptera     | Belostomatidae  | Belostoma     |             |
| Hemiptera     | Corixidae       | Hesperocorixa |             |
| Hemiptera     | Corixidae       | Sigara        |             |
| Hemiptera     | Gerridae        | Aquarius      |             |
| Hemiptera     | Gerridae        | Gerris        |             |
| Hemiptera     | Nepidae         | Ranatra       |             |
| Hemiptera     | Pleidae         | Neoplea       |             |
| Hemiptera     | Veliidae        | Microvelia    |             |
| Hirudinida    | Erpobdellidae   | Dina          | parva       |
| Hirudinida    | Erpobdellidae   | Erpobdella    | punctata    |
| Hirudinea     | Glossiphoniidae | Placobdella   |             |
| Hirudinea     | Glossiphoniidae | Glossiphonia  | complanata  |
| Hirudinea     | Glossiphoniidae | Helobdella    | stagnalis   |
| Hirudinea     | Haemopodidae    | Haemopsis     |             |
| Isopoda       | Asellidae       | Asellus       |             |

|                 |                   |                |  |
|-----------------|-------------------|----------------|--|
| Isopoda         | Asellidae         | Caecidotea     |  |
| Isopoda         | Asellidae         | Lirceus        |  |
| Isopoda         | Oniscidae         | Oniscus        |  |
| Lepidoptera     | Pyralidae         |                |  |
| Mermithida      | Mermithidae       |                |  |
| Odonata         | Aeshnidae         | Aeshna         |  |
| Odonata         | Aeshnidae         | Boyeria        |  |
| Odonata         | Calopterygidae    | Calopteryx     |  |
| Odonata         | Coenagrionidae    |                |  |
| Oligochaeta     |                   |                |  |
| Plecoptera      | Chloroperlidae    | Haploperla     |  |
| Plecoptera      | Perlodidae        | Isoperla       |  |
| Rhynchobdellida | Glossiphoniidae   | Placobdella    |  |
| Sphaeriida      | Sphaeriidae       | Musculium      |  |
| Sphaeriida      | Sphaeriidae       | Pisidium       |  |
| Trichoptera     | Brachycentridae   | Brachycentrus  |  |
| Trichoptera     | Glossosomatidae   | Glossosoma     |  |
| Trichoptera     | Glossosomatidae   | Protoptila     |  |
| Trichoptera     | Hydropsychidae    | Ceratopsyche   |  |
| Trichoptera     | Hydropsychidae    | Cheumatopsyche |  |
| Trichoptera     | Hydropsychidae    | Hydropsyche    |  |
| Trichoptera     | Hydropsychidae    | Parapsyche     |  |
| Trichoptera     | Hydroptilidae     | Hydroptilidae  |  |
| Trichoptera     | Lepidostomatidae  | Lepidostoma    |  |
| Trichoptera     | Leptoceridae      | Oecetis        |  |
| Trichoptera     | Leptoceridae      | Triaenodes     |  |
| Trichoptera     | Limnephilidae     | Anabolia       |  |
| Trichoptera     | Limnephilidae     | Limnephilus    |  |
| Trichoptera     | Limnephilidae     | Pycnopsyche    |  |
| Trichoptera     | Philopotamidae    | Chimarra       |  |
| Trichoptera     | Phryganeidae      | Ptilostomis    |  |
| Trichoptera     | Polycentropodidae | Polycentropus  |  |
| Trichoptera     | Psychomyiidae     | Lype           |  |
| Trichoptera     | Rhyacophilidae    | Rhyacophila    |  |
| Trichoptera     | Thremmatidae      | Neophylax      |  |
| Trombidiformes  | Limnesiidae       | Limnesia       |  |
| Trombidiformes  | Prostigmata       | Hydracarina    |  |
| Trombidiformes  | Sperchontidae     | Sperchon       |  |