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Rush River Macroinvertebrate Monitoring

2023 Sampling Report

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Project Coordinator

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Kiap Tu Wish Chapter of Trout Unlimited
Lake Pepin Legacy Alliance.

Many individuals contributed to the project. Our team of volunteers showed an outstanding commitment to the project and completed the sampling at all 16 sites in less than a month. The sampling team was as follows:

Mitchell Abbett
David Drewiske
Matthew Klein
Carl Nelson
Cynthia Nelson
Mark Peerenboom
James Sauter.

Retta Isaacson of the Pierce County Land Conservation Department provided outstanding support to the team in several areas including expert specimen identification and WAV sampling. We would like to thank Pierce County for their support of the project and the loan of sampling equipment.

Scientific consultants were Michael Miller of the Wisconsin WDNR (WDNR), Kent Johnson of the Kiap Tu Wish Chapter of Trout Unlimited, and Dr. Clarke Garry, Professor Emeritus at UW River Falls. Dr. Kurt Schmude of UW-Superior and the Lake Superior Research Institute performed the laboratory analysis of the samples. The author would like to thank Dr. Schmude (a former student of W.L. Hilsenhoff) for his many informative communications regarding the samples.

Cover Photo: The dolomite outcrop at “Stonehammer” is familiar to many and provides a beautiful setting for one of the sampling sites. Carl Nelson photo.

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Summary

Macroinvertebrates is a term used to describe invertebrates that are large enough to see easily with the naked eye. This includes aquatic insects, crustaceans, mollusks, worms, and a few other types of animals. Since they have widely varying levels of tolerance to pollution, they are good indicators of stream health. Aquatic insects such mayflies, caddisflies, and stoneflies are some of the most sensitive macroinvertebrates, spending most of their lives as larvae that use gills to breathe dissolved oxygen in the streamflow. This critical supply of oxygen is reduced when it is consumed in bacterial decomposition of organic compounds from wastewater, agricultural runoff, and other sources. The results can be lethal for some aquatic insects. Hence, regular monitoring of macroinvertebrate populations is a valuable tool in detecting organic pollution and other stressors. Since 2010 this data has been lacking for the Rush River and its tributaries. The Rush River Macroinvertebrate Monitoring Project is a newly organized volunteer-based effort aimed at gathering, organizing, and communicating macroinvertebrate data on the Rush watershed. During September and October 2023, the project team collected samples at 16 sites along the Rush and three tributaries, Morgan Coulee Creek, Lost Creek, and Cave Creek. We collected and preserved samples according to established protocols (WDNR 2017,) and Dr. Kurt Schmude analyzed the samples in the lab. Our efforts were successful in supplementing the existing body of data on the Rush with a significant amount of new data. The entire length of the river was covered, including sites both with and without historical data.

Many different metrics were obtained for each of the 16 sites, and this report attempts to include as much information as possible for readers who are interested in details. Some high points of the data are the following:

- A vast diversity of macroinvertebrates was found in the riffle habitats on the Rush and its tributaries. A total of 4440 specimens were identified from the 16 samples, with a total of 133 taxa identified. (Note: the term "taxa" (singular "taxon") can indicate any level of classification, but as used here usually indicates species.)
- The macroinvertebrate fauna differed significantly from site to site. There is no typical taxa composition. EPT taxa (mayflies, caddisflies, and stoneflies) made up 50 percent of the specimens identified, although stoneflies were rare.
- Abundant species included the mayfly *Teloganopsis deficiens* (746 specimens), the riffle beetle *Optioservus fastiditus* (622), the mayfly *Baetis tricaudatus* (269), and the two caddisfly species *Ceratopsyche alhedra* (202) and *C. slossonae* (197). Scuds were also abundant at two of the tributary sites.
- Half the sites had a Hilsenhoff Biotic Index (HBI) in the "excellent" range of less than 3.50. At all but two of the remaining sites, this index was in the "very good" range (3.51-4.50).
- Two sites had a more elevated HBI (4.51-5.50). These results are evidence that some organic pollution may be present. These two sites are the farthest downstream and farthest upstream sites, separated by approximately 18 miles.
- The Water Action Volunteer (WAV) index shows a poor correlation with the HBI for both the WAV-1 and WAV-2 indices.

It is important to recognize that environmental factors other than organic pollution can have a significant impact on macroinvertebrate-based metrics. Streambanks erosion results in widening of stream channels and an associated reduction in streamflow velocities. which in turn influences the availability of dissolved oxygen. In addition, an excess of sediment flowing in from both upland sources and eroding banks causes sand and silt to fill the interstitial spaces in coarser gravel and cobble-bottomed streams, reducing habitat quality for both invertebrates and fish.

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The findings outlined above can be combined with both past and future results to study trends. The river is constantly in flux, subject to seasonal and annual variation as well as long-term changes and evolving stresses. Despite our best efforts, collection methods can be inconsistent. With these caveats, it is useful to state some preliminary conclusions which are intended to open up further discussions, avenues of investigation, and plans for future monitoring focused on specific questions and specific locations.

- The middle stretch of the Rush (Hwys 10 to 29) appears to have a healthy macroinvertebrate assemblage indicating likely little or no organic pollution.
- Some organic pollution is likely present on the upper and lower stretches of the Rush. Near term (2024) monitoring efforts should be concentrated on these two stretches. For the three tributaries studied under this project, HBI values indicate little or no organic pollution at present. However, due to low flow, warming temperatures, and agriculture the tributaries are at risk. Future monitoring of Morgan Coulee Creek (a Brook Trout Reserve,) is a priority.
- Coarse-level metrics such as the WAV index, which uses order-level identification of specimens, have the potential to provide timely monitoring without laboratory analysis of samples. It is worthwhile pursuing further use and development of these metrics on the Rush.
- More work is required to study the effects of non-chemical, physical impairments to the stream, such as excessive sedimentation and streambank degradation. In addition, invasive plants (observed at most sampling sites) reduce biodiversity, which may have long-term impacts on the health of the river corridor.

Introduction

The Rush River is a tributary of the Mississippi River lying almost entirely within Pierce County in west central Wisconsin. The river valley is a mosaic of different natural and man-made landscapes: from forested hillsides and dolomite bluffs to agricultural fields to flood plain forests and open wetlands. These landscapes include a variety of natural communities and pockets of relatively undisturbed land. The Rush and its tributary valleys contain a significant fraction of the forested land in Pierce County.

The Rush is located at the northern edge of the Driftless Area. Figure 1 shows the USGS topographic map of the lower Rush, clearly illustrating the highly dissected terrain, with many valleys (coulees) branching off from the river valley, often with further upstream branches. Some of these coulees have small spring-fed streams running year-round, while others have dry stream beds except during heavy rains and snow melt. The deeply incised Morgan Coulee, where one of the study tributaries is located, is visible in the center of the map.

Figure 2 shows the river from its source near Interstate 94 to the mouth at Lake Pepin. The Rush drains much of the central portion of Pierce County. It is a Class 1 trout stream with a self-sustaining wild trout population and has two tributaries, Lost Creek and Cave Creek, which are also Class 1. Morgan Coulee Creek is classified as Class 2, although this stream is a WDNR-identified Brook Trout Nursery.

Macroinvertebrate monitoring, as well as habitat assessment and electro-shocking fisheries surveys are used to evaluate stream health. However, a comprehensive assessment of the Rush River watershed has not been published by the WDNR in more than two decades (Engel and Michalek 2002.) Fisheries Surveys of the Rush River (Yallaly 2021) are carried out on a regular basis. These surveys show that the Rush continues to support a healthy trout population. Superlatives are often used to describe the productivity of the fishery. However, fisheries surveys do not provide data on the biotic community that trout depend upon for food and is subjected to stresses that may not be apparent. This can only be obtained through regular and rigorous monitoring of macroinvertebrates. While macroinvertebrate sampling and analysis was routinely carried out by the WDNR in the past, this activity has been curtailed since at least 2018, due to higher priority being given to impaired waters. The purpose of the Rush River Macroinvertebrate Monitoring Project is to carry on this activity with volunteers and ensure that new data are made available on a timely basis. This will be included and preserved within the WDNR's Surface Water Integrated Monitoring System (SWIMS) database, for use in documenting and understanding the invertebrate assemblage. Given the documented decline (Houghton and DeWalt 2023, O'Harrow 2022) in the population of key aquatic insect species in the orders Ephemeroptera (mayflies,) Trichoptera (caddisflies,) and Plecoptera (stoneflies,) this study has taken on added urgency.

Figure 1. USGS topographic map of the lower Rush River. Heavy red line at top is State Highway 10.

Figure 2. The Rush River and surrounding area. Class 1 trout streams in green. Source -WDNR.

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Project Sampling Sites

The team carried out macroinvertebrate sampling at a total of 16 sites as follows:

1. 10 sites on the Rush River, beginning at the 385th St bridge, three miles north of Hwy 35, and ending at St. Croix County Rd Y around 18 miles to the north.
2. Two sites each on the Class I tributaries Lost Creek and Cave Creek. Site 10 is a new SWIMS site added for this project.
3. Two sites on the Class II tributary Morgan Coulee Creek.

Table I shows the details of the sites, including latitude and longitude. For historical consistency, all sampling sites were selected to coincide with an existing SWIMS Station ID. Note that all data is associated with the SWIMS Station listed. Latitude and longitude were copied directly from the SWIMS database for the Station ID. The exact sampling location was as close to this as possible, given the stream conditions, accessibility, and the location of suitable riffle habitat. The exact lat/long where sampling occurred was measured with a GPS unit and recorded on the WDNR Wadeable Macroinvertebrate Field Data Report, Form 3200-081 (R 08/14) (the "Labslip") completed for each sample.

Site selection was based on a number of factors, including: 1) coverage of the entire Rush River and its important tributaries, 2) sampling at sites with historical macroinvertebrate sampling. Of the 16 proposed sites, nine have existing macroinvertebrate data and associated metrics, including HBI. 3) sampling at selected stations used for Fisheries Surveys. Figure 3 shows the site locations. Note that the sites are assigned an additional identifier 1-16 in order of increasing latitude.

Table 1. Sampling Sites

SWIMS Station ID in parentheses, Fisheries Surveys Station Number in square brackets.

Number	Name	Latitude	Longitude
1	Morgan Coulee Cr - 385th St [1] (Station 10008810)	44.61012	-92.32054
2	Morgan Coulee Cr - 200th Ave [2] (Station 10008820)	44.61121	-92.30159
3	Rush River - 385th St [4] (Station 10008903)	44.62715	-92.33186
4	Rush River - 2000m south of HWY 10 Bridge (Station 10029204)	44.65543	-92.32250
5	Rush River - 450th Avenue [8] (Station 10008913)	44.69481	-92.32974
6	Lost Creek - 465th Ave [1] (Station 483083)	44.70186	-92.33180
7	Lost Creek - 450th St [3] (Station 10008892)	44.71171	-92.36061
8	Cave Creek - Hwy 72 [1] - 1 Mi East Of BB (Station 483037)	44.73325	-92.30824
9	Rush River - Hwy 72 [10] - NRSA Site (Station 10051363)	44.73351	-92.32630
10	Cave Creek - 610th Ave (Station 100574630	44.75742	-92.30099
11	Rush River - Hwy N El Paso (Station 483078)	44.77197	-92.34383
12	Rush River - Stonehammer [14] (Station 10008914)	44.78906	-92.36605
13	Rush River - Wonderland [15] - 50' below mouth of Gilman Cr (Station 10044498)	44.80797	-92.37478
14	Rush River - Hwy 63 Martell [16] (Station 10008924)	44.82948	-92.39459
15	Rush River - Hwy 29 [17] (Station 10008922)	44.84811	-92.40194
16	Rush River - CTH Y [18] (Station 10008918)	44.86821	-92.40884

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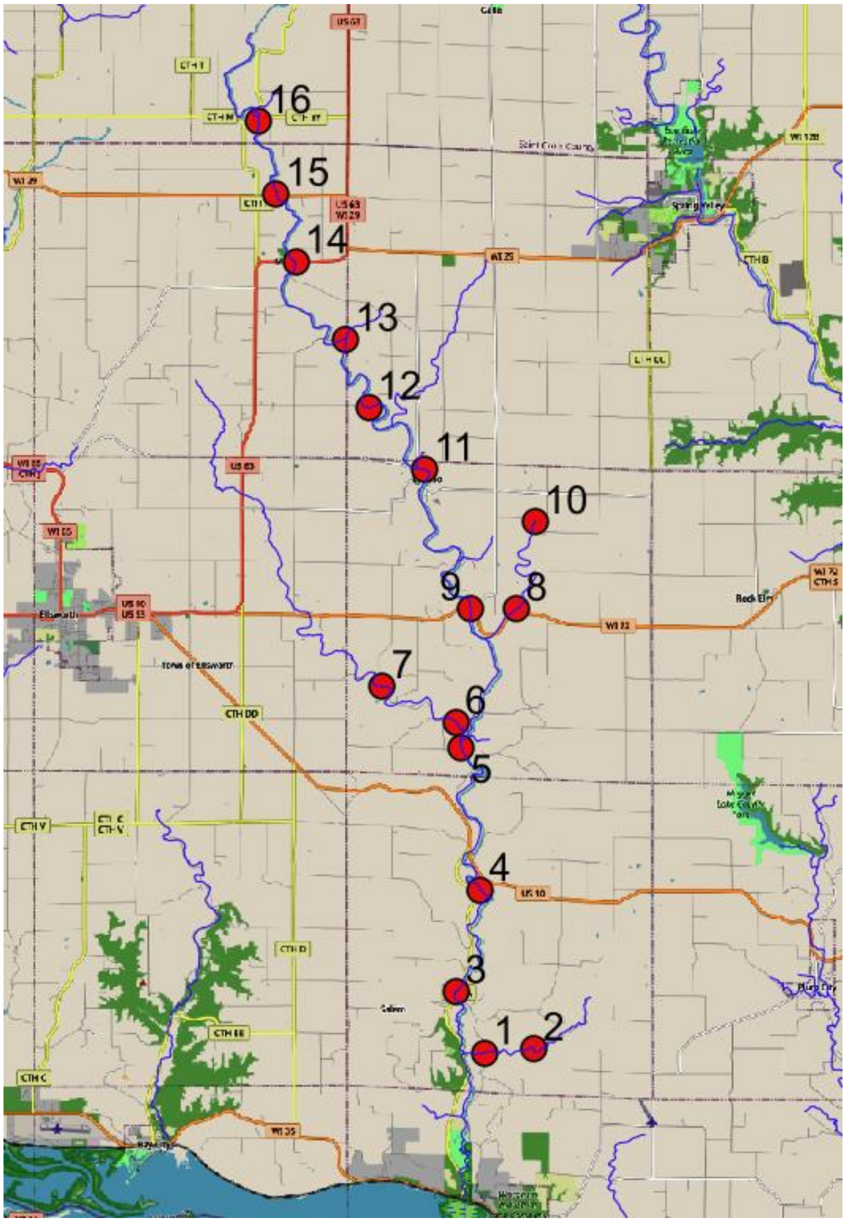

Figure 3: Sampling sites. Map created in QGIS Desktop 3.24.1.

The Surface Water Data Viewer (SWDV) (https://dnrmaps.wi.gov/H5/?Viewer=SWDV) was the primary tool used to select these sites. This software contains the location of all sampling sites where any type of data has been collected going back as far as 1979, as well as links to the actual data sets, including water quality, invasive species observations, and invertebrate sampling and analysis.

Sampling Methodology

The sampling method is adapted from the WDNR document “Guidelines for the Standard Collection of Macroinvertebrate Samples from Wadeable Streams v2.0,” (WDNR 2017). This document specifies sampling over approximately a 0.5 m^2 area for 1-2 minutes for each sample. Our project methodology utilized a composite sample collected over three transects, each approximately 1/3 m^2, with a sample time of 30 seconds for each transect. All samples were taken in riffles.

WDNR 2017 provides detailed descriptions of the collection method, sample preservation, and sample labeling requirements. Sampling labeling requires the use of two labels attached to each sample, one inside and one outside the sample jar. All macroinvertebrate samples also need to have a macroinvertebrate labslip generated from the SWIMS database before they can be submitted to a WDNR approved lab (UW-Superior) for taxonomic identification, which are associated with a new or existing SWIMS project. A SWIMS project entitled “Rush River Macroinvertebrate Monitoring Project” has been created.

The sampling methodology is described below.

Sampling equipment:

- 1 waterproof site-specific Labslip/Field Data Report form per site
- 1 labeled wide mouth, 1-litre HDPE sample jar per site, with waterproof label taped on
- 1 additional waterproof copy of label for placement inside the jar
- 80% and 95% ethanol, denatured
- D-frame net–500 micron mesh
- Tweezers for removing specimens from net, pipettes, magnifying glass, spoons
- 35 Mesh (500 micron) sieve
- 2 Plastic sample tubs (white)
- A small bucket for collecting stream water
- Measuring container
- Squirt bottles – one with water, one with 80% ethanol
- Meter measuring stick
- 6 flags to mark 3 transects for kick sites
- Garmin GPS
- Marsh McBirney Velocity Meter
- Tape measure and anchors
- Spherical densiometer for canopy cover measurement
- Stopwatch/phone/watch to time sample collection period
- Pencils to write on waterproof forms
- Clipboards
- Waders

Project standardization of effort

Three kick samples were taken per site and combined into a composite sample in the sample jar. Sampling time was 30 seconds per kick. Samples may be contained in a debris ball approximately softball size.

Sampling Procedure

1. **Double check** that SWIMS Station information and Sample ID on the labslip are correct and match the **SWIMS Station ID** and **Sample ID** on the labels. Tape one label to the sample jar.
2. **Set up collection equipment**.
3. **Time and process**: On the labslip, note beginning and ending time at each site.
4. **Determine 3 kick sites (transects) for lab samples**. Mark with flags 1 meter apart. Sample the most downstream site first. Do not step in marked kick sites!
5. **Kicking method and collection into sample jar**: For each transect, place net on stream bottom even with downstream flag, holding the net frame firmly against the stream bottom. Perform kicking in an area approximately one meter long (the length of the transect) and slightly wider than the kick net. The area should be approx. one third of a square meter. Use the toe or heel of your wading boot to disturb the stream sediment. Kick deeply to dislodge macroinvertebrates attached to rocks, debris and those in the first few centimeters of sediment.
    a. You should see a plume of debris (mostly silt) and be sure the current is washing this plume into your kick net.
    b. Continue this process for 30 seconds.
    c. If fine sediment is present in the sample debris ball, sweep the kick net upstream a number of times to sieve out any silt. Be sure not to lose the contents of the sample while conducting this procedure. Remove any coarse debris. While still inside the kick net, large rocks, sticks, and plant material should be vigorously rinsed to dislodge any macroinvertebrates and then discarded.
    d. Invert the net and place all the contents of the net into first sample tub, using wash water rinse from the inside and using tweezers collect all invertebrates still on the side of the net. Transfer all the contents of the sample tub into the 35-mesh sieve.
    e. Fill second sample tub with fresh water and dip sieve in tub to wash out any fine particles. Then transfer all contents of the sieve into the sample jar. Rinse sieve with 80% alcohol from squirt bottle to wash all macroinvertebrates into the sample jar.
    f. Repeat the above for the other two transects, adding to the same sample jar. This should result in ~1.0 square meter sampled. Sample jar should be filled with 80% alcohol, and not more than half full of debris.
6. **Sample Preservation**: Add 80% ethanol directly to the sample jar to fill it. Insert the duplicate label inside the sample jar. Close the container and **gently** invert multiple times to distribute.
7. **Complete Sample and Site Descriptors** on labslip.
8. **Within 48 hours, decant and re-preserve sample per WDNR 2017**. Pour-off the alcohol solution and refill with fresh 95% ethanol. Replace label in jar. Use electrical tape to seal lid closed. Samples containing large amounts of organic materials should be preserved and re-preserved several times. (This was not necessary for the current samples.)

Figures 4-8 show the sampling equipment, sampling sites, and the volunteers in the field.

Figure 4. Three transects for sampling at Rush River 450th Av, Station 10008913.

Figure 5. 35 Mesh (500 micron) sieve, sample at Morgan Coulee Creek, 385th St, Station 10008810.

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Figure 6. Dave Drewiske and Matt Klein at Rush River, HWY 29, Station 10008922.

Figure 7. Mitch Abbett and Jim Sauter at Lost Creek, 465^{h} Av, Station 10008810.

Figure 8. Sample jars from 11 sites, ready for delivery to lab.

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Sampling Results

Dr. Kurt Schmude, Professor in the Department of Natural Sciences and Senior Scientist at the Lake Superior Research Institute, University of Wisconsin-Superior, analyzed all 16 samples. Specimens were identified to the species level whenever possible. The results were entered into the SWIMS database, along with all information recorded on the labslip. For each sample, macroinvertebrate specimens were identified in randomly selected grids until at least 250 specimens were obtained, counting all the specimens in the selected grid.

Taxa Analysis (Class/Order/Family Level) by Site

The laboratory analysis identified between 250 and 310 total specimens for each sample, as described above. A useful, high-level picture of the macroinvertebrate community at the sample site is provided by the percentage of specimens belonging to various taxa. The taxa represented in the samples are listed below. For completeness, all taxa are listed, although some were represented by only a few specimens.

In this list, macroinvertebrates are generally classified according to Order (bold face). For the Order Diptera, a further classification into families is given, with Chironomidae (midges) being the most numerous. For most non-insect macroinvertebrates, a higher classification (phylum or class) is listed.

Ephemeroptera-Mayflies
Plecoptera-Stoneflies
Trichoptera-Caddisflies
(Note: The above three orders are commonly grouped together
and abbreviated by **EPT**)
Megaloptera-Alderflies, Dobsonflies, and Fishflies
Coleoptera-Beetles
  - Elmidae-Riffle Beetles.

Hemiptera-True Bugs
  - (Two species were represented)
  - Belostoma flumineum-Giant Water Bug
  - Sigara trilineata-Water boatmen

Diptera-Flies
  - Chironomidae-Midges
  - Simuliidae-Black Flies
  - Tipulidae-Crane Flies

Amphipoda-Scuds
Hydrachnidia-Mites
Oligochaeta (Class)-Worms
Turbellaria (Class)-Flatworms
Nemertea (Phylum)-Proboscis worms
Hirudinea (Class)-Leeches
Gastropoda (Class)-Snails
Bivalvia (Class)-Fingernail Clams

Figures 9 and 10 show the taxa composition for each of the 16 sites. These charts show that the macroinvertebrate faunal composition differed significantly from site to site. There is no typical taxa composition. For the Rush River sites, the mayfly percent varied from a low of 9% at site 16 to a high of 60% at site 9 (Hwy 72 in Martell.) Caddisfly percentage ranged from a low of 3% at site 3 (385th St) to 50% at site 16. Stoneflies were 1% or less at all sites on the Rush. Riffle beetles, *Optioservus fastiditus*, made up a significant part of the taxa at all Rush sites except for site 3, where the riffles were weak and not well oxygenated. By contrast, at site 4 (2000m S of Hwy 10) riffle beetles accounted for almost half (45%) of the specimens identified. Scuds were significant (5%) only at site 3.

For the three tributaries (6 sites,) the mayfly percent varied from a low of 2% at site 7 on Lost Creek to a high of 25% at site 8 on Cave Creek. Caddisflies numbers were relatively consistent, ranging from a l4% at site 10 to 28% at site 8, both on Cave Creek. Stoneflies were absent on Morgan Coulee Creek and found in significant numbers (10%) at only one tributary site (site 6 on Lost Creek). Riffle beetles ranged from 1% at site 8 (Cave Creek Hwy 72) to 23% at site 1. Scuds were very abundant (35%) at one site, Site 1 on Morgan Coulee Creek, while much less abundant (5%) upstream at site 2. Scuds were found in significant numbers (9%) at one other site, site 10 in the upstream reaches of Cave Creek. This was also the only site where snails were significant. Worms were generally more abundant in the tributaries than in the main river.

Appendix A gives a complete list of all species for all sites. This allows a more detailed analysis of the taxa, and the examination of various parameters that may provide information about stream health. A few of these are listed below:

1. The species diversity (richness) for each site and over all sites for Ephemeroptera (mayflies,) Trichoptera (caddisflies,) and Plecoptera (stoneflies) should be examined. For example, the caddisfly species richness in the Rush River is less than 10 species at any given site and 20 species over all sites. A priority is to determine whether this result is “normal” or indicates declining stream health. Houghton and DeWalt (2023) state that least disturbed streams generally average around 30 species, while highly disturbed streams 6may have as few as 10 species. However,, they also predict that Driftless Area streams will have low caddisfly species richness even when undisturbed. The majority of caddisflies are in the family Hydropsychidae, genus *Ceratopsyche*. See Schmude and Hilsenhoff (1986) for a discussion of this family.

2. The presence/absence/abundance of a particular species at a given site, especially species with low pollution tolerance, may serve as critical indicators for stream health. For example, the *Emphemerella excrucians* mayfly was found in moderate numbers (11 specimens) at Site 4 on the Rush. The historical trends for a particular species are of vital importance. For example, *Emphemerella excrucians* mayfly was more abundant in the 2004 sampling at this site. The lower numbers of this species in the 2023 sampling contributed to a significant increase in the HBI index at this site, as discussed in the next section. It is relevant that Bruce Markert (Personal communication, 2024) reports that this species, which has low pollution tolerance, has disappeared from Black Earth Creek in Dane County.

   The caddisfly *Brachycentrus occidentalis* is another potential indicator species with very low pollution tolerance. The presence of these two species in significant numbers, as well as the *Baetis tricaudatus* mayfly, are primary contributors to a low value (low is good) of the Hilsenhoff Biotic Index (HBI) that will be discussed in the next section.

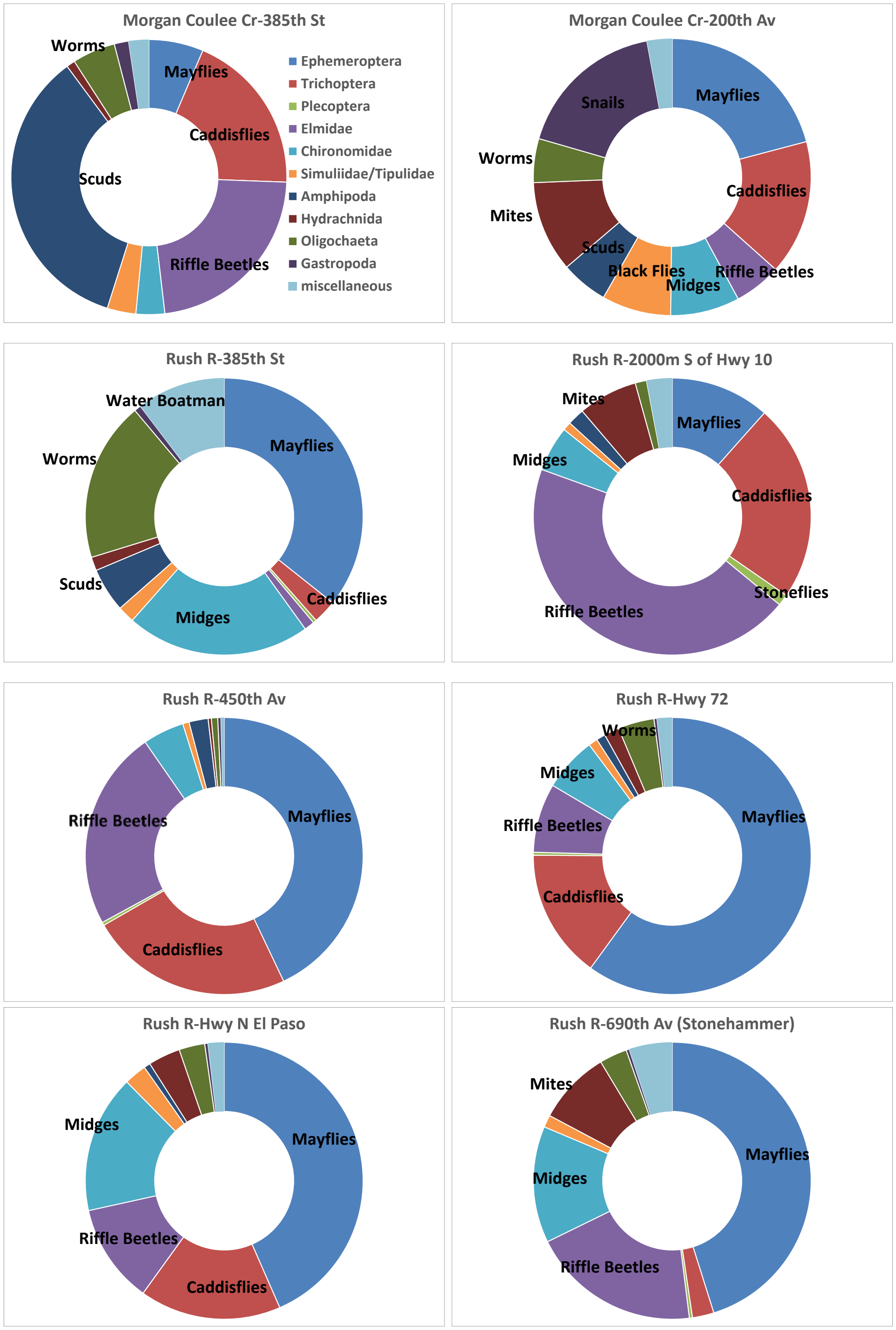

Figure 9. Percent taxon, Morgan Coulee Creek and Rush River to Stonehammer.

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Figure 10. Percent taxon, Rush River Wonderland to Site 16, Lost Creek and Cave Creek.

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A total of 4440 specimens were identified from the 16 samples, with a total of 133 taxa identified. Appendix B gives a list of taxa ranked by total count over all sites. Abundant species included the mayfly *Teloganopsis deficiens* (746 specimens), the riffle beetle *Optioservus fastiditus* (622), the mayfly *Baetis tricaudatus* (269), and the two caddisfly species *Ceratopsyche alhedra* (202) and *C. slossonae* (197). Scuds were also abundant at two of the tributary sites.

A useful coarse-level metric that can be obtained from the taxa list is the combined total number EPT specimens as a percentage of the total specimens identified for a sample. Since taxa belonging to the EPT orders (mayflies, stoneflies, and caddisflies) are generally the most sensitive to organic pollution, a high EPT percentage indicates good stream health. Figure 11 shows this metric for the Rush and the three tributaries, with EPT percent plotted versus site latitude. Since latitude measures distance upstream in the watershed, this figure shows that EPT percent was less than 40 in the lower watershed, increased sharply above Highway 10, then decreased somewhat in the upper watershed above Highway 72. It reached a maximum value of 75 percent at the Highway 72 site. In the tributaries, EPT percent was less than 60 percent at all sites. It reached a low of 26 percent at Site 1 on Morgan Coulee Creek. A sharp decrease was seen on Lost Creek between the downstream site and upstream site, where there was a low percentage of mayflies.

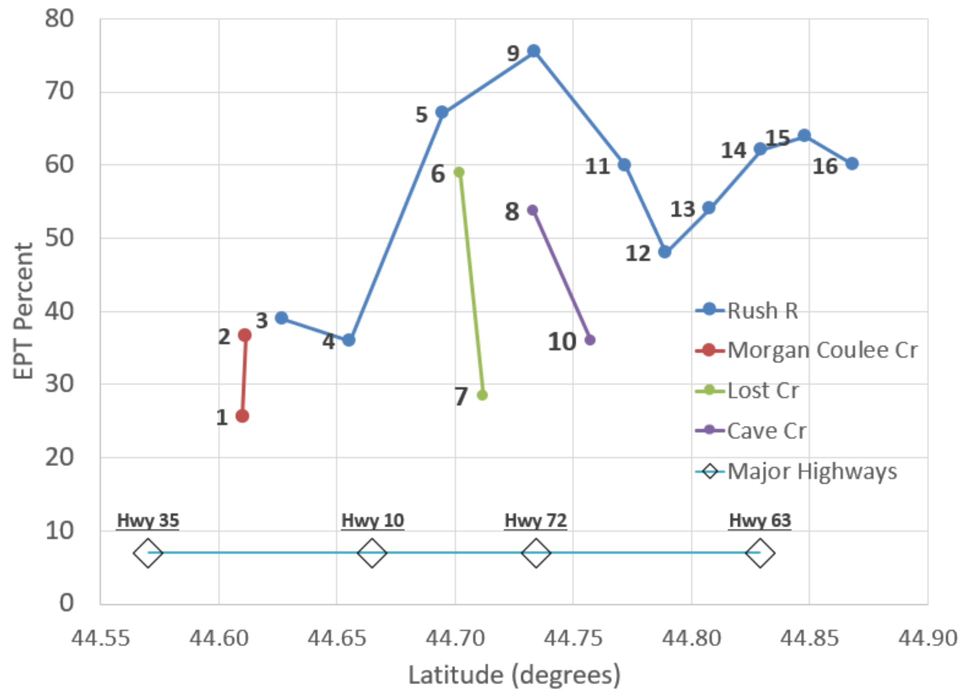

Figure 11. EPT percent vs. sample site latitude. See Table 1 for site details. EPT percent over all sites was 50.3%.

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Figure 12 plots two additional metrics related to taxa richness. Note that these are obtained by counting taxon occurrences, and a single individual may be counted as an occurrence. Both EPT and total taxa richness are shown in this figure. These plots show a relatively narrow range for each of these metrics, 5 to 18 for EPT, 27 to 49 for total taxa richness. In both cases the three tributaries were generally within the same range as the main river. The high of 49 total taxa was recorded for Site 6 on Lost Creek. The low of five EPT taxa was recorded for the upstream site (Site 10) on Cave Creek. Note that in some cases (especially for the chironomids), a single taxon may include more than one species. This might occur when specimens cannot be identified to the species level.

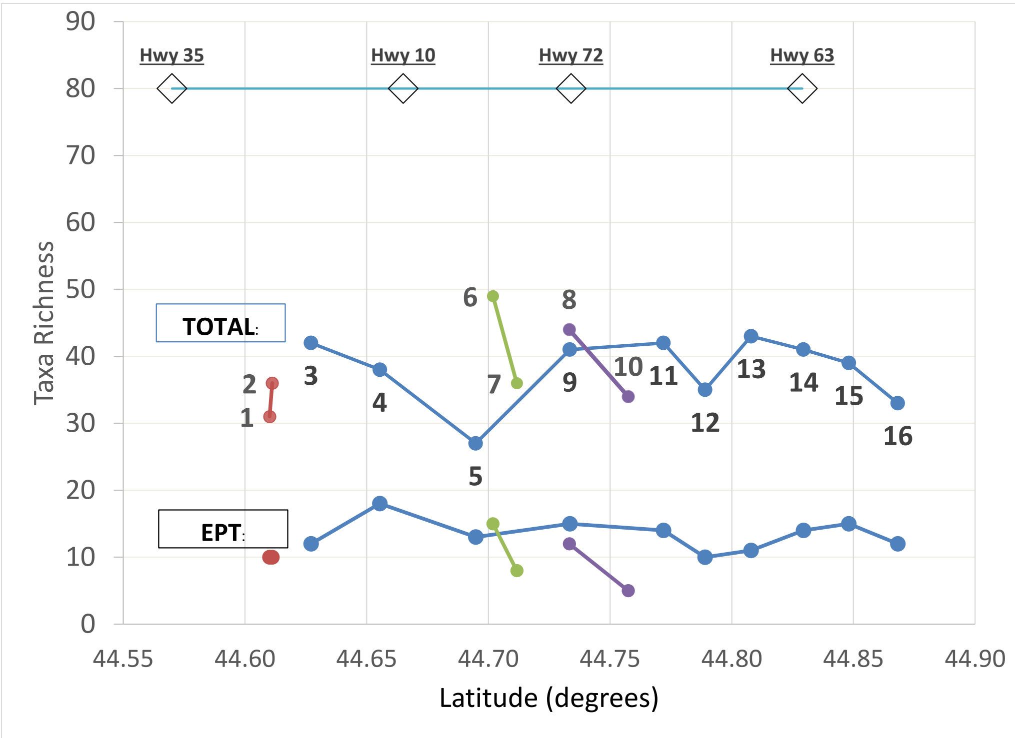

Figure 12. EPT (bottom curves) and total (top curves) taxa richness vs. latitude. See Fig. 11 for legend.

Biotic Indices

The use of biotic indices to evaluate water quality in Wisconsin streams has a long history beginning with the work of W.L. Hilsenhoff (1982, 1987). Hilsenhoff assigned organic pollution tolerance values to various macroinvertebrates at the species and genus level, initially 1-5 (1982) and later revised to 1-10 (1987) Note that tolerance values increase with increasing levels of tolerance. The Hilsenhoff Biotic Index (HBI) is the weighted average, computed for each sample, of these tolerance values over all the specimens identified using the random subsampling technique discussed above. The equation, in Hilsenhoff's notation (1982) is

$$HBI = \sum \frac{n_i\, a_i}{N}$$

Where n_i is the number of each species (or genus) identified in the sample, a_i is the tolerance value assigned to that species (or genus), and N is the total number of individuals in the sample, i.e. $N = \sum n_i$.

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Table 2 shows the pollution tolerance values for the six most abundant species found over all the samples, as listed in decreasing order of abundance in Appendix B.

Table 2. Pollution Tolerance Values for Six Most Abundant Species

Rank	Latin Name (genus/species)	Order/Family	Common Name	Tol. Value
1	*Teloganopsis deficiens*	Ephemeroptera/Ephemerellidae	Little Black Quill	2
2	*Optioservus fastiditus*	Coleoptera/Elmidae	Riffle Beetle	4
3	*Baetis tricaudatus*	Ephemeroptera/Baetidae	Blue Wing Olive	2
4	*Ceratopsyche alhedra*	Trichoptera/Hydropsychidae	Common Netspinner	3
5	*Ceratopsyche slossonae*	Trichoptera/Hydropsychidae	Common Netspinner	4
6	*Gammarus pseudolimnaeus*	Amphiboda/Gammaridae	Scud	4

The tolerance values in the table above can be interpreted in the context of Hilsenhoff's water quality rating system, shown Table 3. Thus, if the sample has an abundance of species with a tolerance value of less than 4, we can expect the weighted average of tolerance values (i.e. the HBI) to be in the "excellent" range (< 3.50). Conversely if species with tolerance values greater than 4 dominate the sample, we can expect the HBI to be significantly higher and possibly in the "good" range or lower. However, the ability of the HBI to discriminate among seven levels of water quality based upon the somewhat arbitrarily defined ranges in Table 3 is questionable. Therefore, the HBI should be regarded as a continuum of values rather than a set discrete "ratings."

Table 3. Water Quality Rating for Hilsenhoff Biotic Index (HBI) From Hilsenhoff (1987)

HBI Value	Water Quality Rating	Degree of Organic Pollution
< 3.50	Excellent	None Apparent
3.51 – 4.50	Very Good	Possible Slight
4.51 – 5.50	Good	Some
5.51 – 6.50	Fair	Fairly Significant
6.51 – 7.50	Fairly Poor	Significant
7.51 – 8.50	Poor	Very Significant
8.51 – 10.00	Very Poor	Severe

Figure 13 shows the HBI versus latitude for the Rush and its tributaries. The HBI values range between from a low of 2.92 at Site 9 (Hwy 72) to a high of 5.01 at Site 1 at 385th St on the lower River. For the tributaries, HBI values lie in a narrower range from 3.10 to 4.06. Half of the 16 sites have an HBI less than 3.50 ("excellent,") and all but two of the remaining sites are only slightly higher ("very good.") The lowest values of HBI are generally related to a high percentage of mayflies. The exception to this is Site 6 on Lost Creek where an abundance of *Brachycentrus* caddisflies (tolerance value of 1) accounts for a low HBI.

The two sites with the highest HBI are at the two geographical extremes, i.e. the farthest downstream and the farthest upstream sites on the Rush. These are close to 5.0 ("good,") indicating some organic pollution. The elevated HBI at the downstream site (Site 3) can be traced to an abundance of midges along with more tolerant *Baetis* mayflies.

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This site is visibly degraded, with severe bank aggradation and sedimentation. Unfortunately, these conditions are characteristic of much of the lower Rush. The author (Nelson, 2019) in a report entitled "The Lower Rush River: Present Health and a Call to Action" gives a detailed discussion of the lower river. As a footnote, On September 29, 2023 the monitoring team observed another problem when sampling at Sites 3 and 4. Appendix C shows photographs documenting this event. An unusual discoloration of the streamflow at Site 3 was observed, and when the team moved to Site 4 upstream, we found a heavy milky discharge from a small intermittent tributary. This was subsequently traced to the Wieser Concrete plant on Hwy 10. A similar discharge following a rainfall event was observed at the same location on October 13, 2023. The DNR has reportedly identified the source within the plant and is currently working with Wieser to correct it.

At the upstream site (Site 16) we found the tolerant *Ceratopsyche morosa bifida* caddisflies to be abundant, resulting in an elevated HBI. Historical data are available for comparison for both sites (3 and 16). This is discussed in the next section.

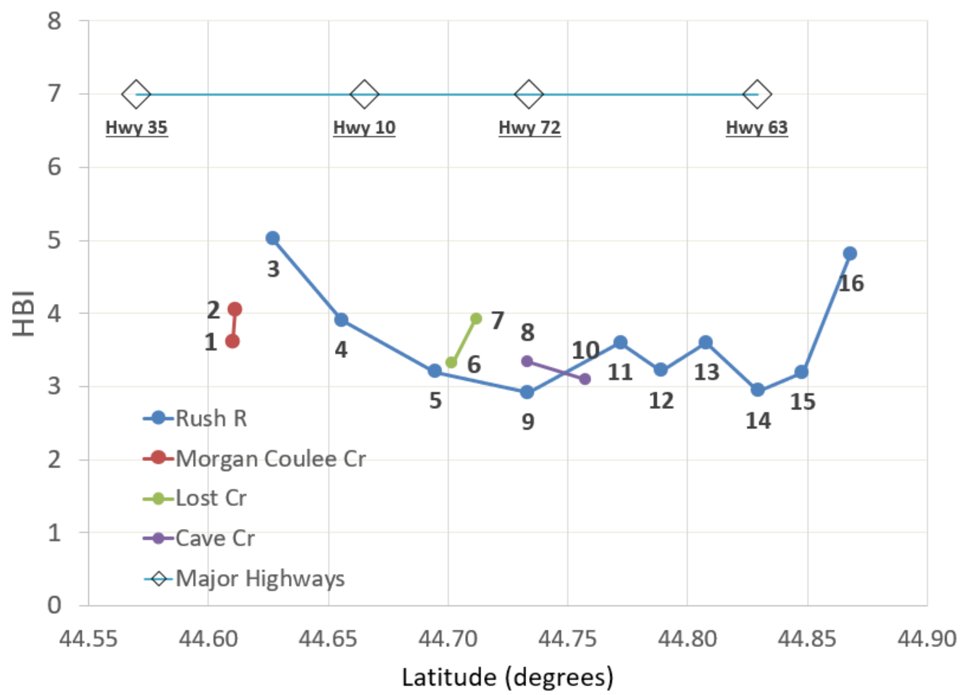

Figure 13. Hilsenhoff Biotic Index vs. latitude.

Hilsenhoff (1988) also added a family-level biotic index (FBI) in 1988 "to provide a rapid, but less critical, evaluation of streams in the field by biologists who can recognize arthropod families by sight." Hilsenhoff noted that the FBI is usually higher (i.e. indicates greater pollution) than the HBI in unpolluted or slightly polluted streams, and lower (indicates less pollution) in polluted streams.

Table 4 shows the FBI values, along with a summary of other metrics. A comparison of FBI with HBI values shows that the FBI differs significantly from the HBI in many cases. Hilsenhoff (1988) gives an FBI-based rating system that is slightly different from the one shown in Table 3 for the HBI. The water quality rating is shown for both HBI and FBI, and it is seen that the FBI rating is better than HBI in four cases, worse in two cases, and the same in 10

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cases. Hence, less or the same level of pollution is indicated by the FBI than the HBI in 14 of the 16 samples (88 percent), contrary to Hilsenhoff's observation. EPT percent appears to have a reasonable correlation with HBI (Figure 14,) but the two sites with the worst water quality rating (3 and 16) are "outliers."

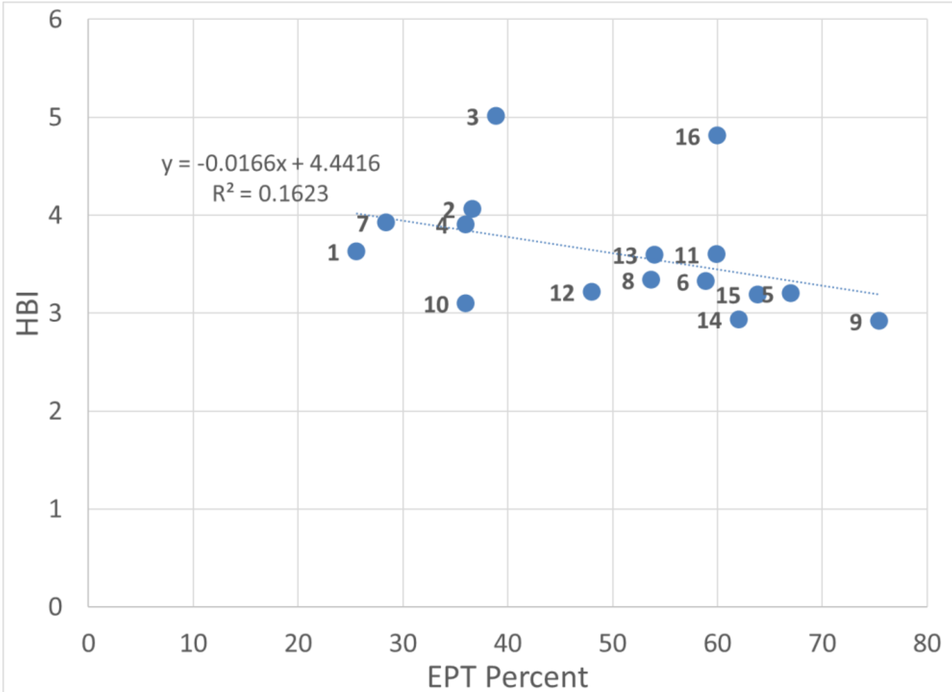

Figure 14. HBI vs. EPT Percent.

Table 4. Summary of Metrics

Site	Description	Latitude	EPT %	HBI	HBI Rating	FBI	FBI Rating	Temp (^{o}C)
0	Hwy 35	44.57	-	-	-	-	-	-
1	Morgan Coulee Cr - 385th St	44.61012	25.59	3.63	Very Good	3.69	Excellent	13.3
2	Morgan Coulee Cr - 200th Ave	44.61121	36.63	4.06	Very Good	4.23	Very Good	11.4
3	Rush River - 385th St	44.62715	38.89	5.01	Good	4.45	Good	13.6
4	Rush River - 2000m south of HWY 10	44.65543	35.97	3.90	Very Good	3.81	Very Good	14.2
5	Rush River - 450th Ave	44.69481	67.04	3.20	Excellent	2.94	Excellent	16.4
6	Lost Creek - 465th Ave	44.70186	58.94	3.32	Excellent	3.63	Excellent	7.0
7	Lost Creek - 450th St	44.71171	28.40	3.92	Very Good	4.13	Very Good	9.0
8	Cave Creek - Hwy 72	44.73325	53.71	3.34	Excellent	4.29	Good	8.9
9	Rush River - Hwy 72 - NRSA Site	44.73351	75.44	2.92	Excellent	2.72	Excellent	9.7
10	Cave Creek - 610th Ave	44.75742	36.01	3.10	Excellent	4.09	Very Good	9.0
11	Rush River - Hwy N El Paso	44.77197	59.93	3.60	Very Good	3.32	Excellent	10.5
12	Rush River - Stonehammer	44.78906	48.03	3.22	Excellent	2.70	Excellent	12.7
13	Rush River - Wonderland	44.80797	54.04	3.60	Very Good	3.05	Excellent	13.5
14	Rush River - Hwy 63 Martell	44.82948	62.07	2.94	Excellent	2.44	Excellent	15.0
15	Rush River - Hwy 29	44.84811	63.87	3.19	Excellent	3.08	Excellent	18.6
16	Rush River - CTH Y	44.86821	60.00	4.81	Good	4.04	Very Good	17.7

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Many other indices are used for various purposes in the evaluation of stream health. Lillie et al. (2003) provides extensive guidelines for macroinvertebrate data interpretation, comparison of the different biotic indices, and the use of the WDNR electronic database.

Water Temperature and Stream Gradient

Water temperature at time of sampling is also included in Table 4. Along with organic pollution, water temperature is an important factor in dissolved oxygen available to macroinvertebrates. The Rush and its tributaries are known to have significant groundwater inflow that contributes to a favorable temperature regime year-round. Measurements taken during sampling show that temperature ranged from 7.0ºC (45ºF) at Site 6 on Lost Creek to 18.6ºC (65ºF) at Site 15 on the Rush at Hwy 29. Water temperature versus site latitude is plotted in Figure 15. Lower temperatures were recorded in the middle stretch of the Rush, along with Lost Creek and Cave Creek. The temperature peaks above Hwy 10 at Site 5, then decreases sharply at Site 9, where the lowest HBI was obtained. The highest temperatures were recorded above Hwy 63.

Since the highest water temperature during summer is critical to stream health, more data on temperature over an extended duration including the summer months is needed. This is a high priority for Morgan Coulee Creek, where high summer air temperatures combined with low flow could pose a threat to the brook trout nursery.

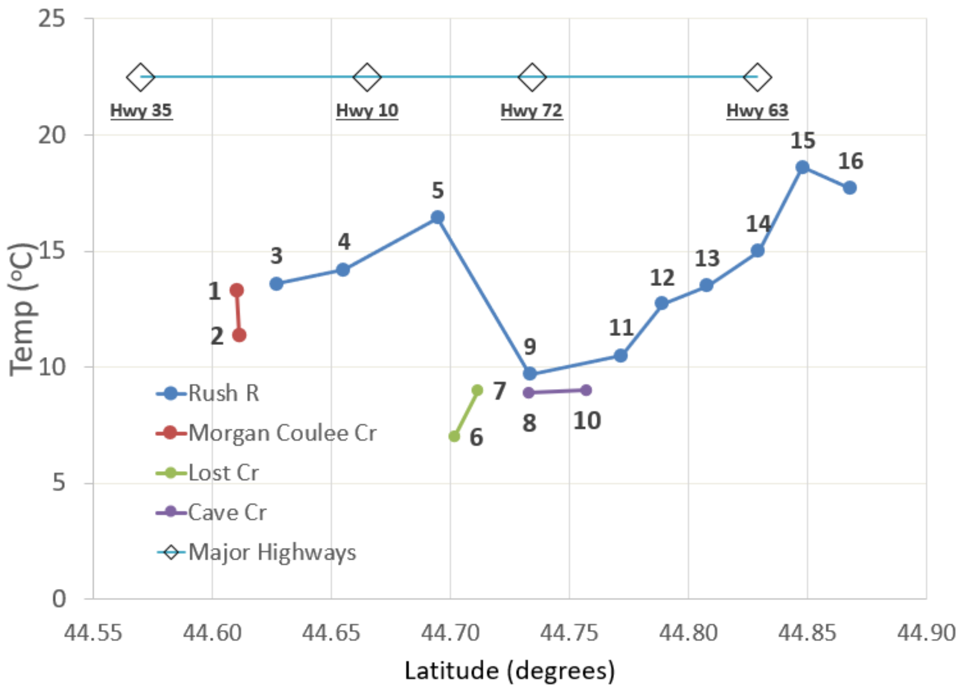

Figure 15. Water temperature at sampling vs. latitude.

Stream gradient determines velocity, which is another critical factor in dissolved oxygen and cold-water habitat. Figure 16 shows the elevation above sea level for the 10 sites on the Rush (along with a baseline location at Highway 35,) plotted versus latitude. Note that an accurate representation of stream gradient cannot be obtained from this data, since this can only be computed by combining a digital description of the exact course of the river, including meanders, with a digital elevation model. (The author is working on this!) However, the slope of the elevation profile shown in Figure 16 provides a rough estimate of the variation of the gradient along the length of the river. The elevation profile exhibits an S-shape, indicating that the gradient increases from its mouth to a maximum north of Hwy 72, then decreases in the upper stretch above Hwy 63. The gradient in the steep middle section appears to be around twice that in the upper and lower sections. This must be verified with a rigorous digital analysis.

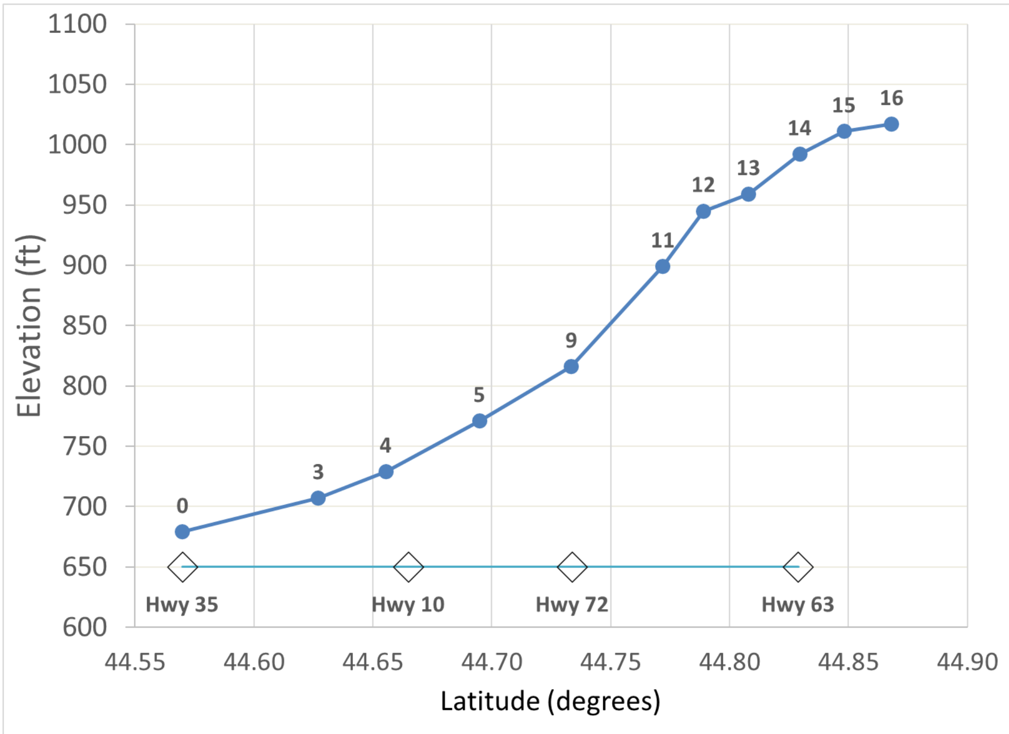

Figure 16. Site elevation (Rush River only) vs. latitude.

WAV Sampling and Analysis Summary

The Water Action Volunteer (WAV) sampling protocol and coarse-level metric is used widely to obtain information on stream health. The WAV index utilizes Order and Class level identifications, which can be made in the field by trained volunteers. Specimens are collected and placed into four groups, according to pollution tolerance, with each group assigned a weight factor. A weighted average of pollution tolerance is computed to obtain the WAV index. In contrast with the biotic indices such as HBI and FBI, the weight factor increases with decreasing pollution tolerance, and consequently the WAV index increases with better stream health. Since the analysis depends only on the presence of a particular organism, and not on the total specimen count from a large sample, the WAV index can only provide a very coarse level of evaluation of the macroinvertebrate fauna represented in the sample.

Recently, Michael Miller, WDNR Stream Ecologist, proposed a modification of the WAV metric. To distinguish it from the current WAV index, these two indices are referred to as WAV-1and WAV-2 respectively. WAV-2 incorporates two major changes. First, the invertebrate groups are redefined, with among other changes, mayflies, caddisflies, and stoneflies all being placed in Group 1 (the most intolerant category.) Second, the WAV-2 index includes a +1 addition for the group with the "most common animal," as determined in the field by an overall impression of the sample collected. The WAV-2 recording form is included in Appendix D.

We carried out both WAV-1 and WAV-2 sampling and analysis in parallel with the main effort described in the previous sections of this report. The specimens required for WAV-1 and WAV-2 sorting were selected from the main sample, with like specimens being placed in separate compartments of an ice cube tray. After the results were recorded, the tray contents were recombined with the original sample for preservation and prepared for transportation for lab analysis. Photographs of the sorting tray were taken to document identification of specimens. See Figures 16 and 17.

Figure 16. Retta Isaacson works on the WAV sorting at Site 4.

Figure 17. WAV sorting tray with specimens at Site 11 in El Paso.

WAV-1 and WAV-2 Results

The WAV-1 and WAV-2 computations are given in Appendix E. Following computations in the field and subsequently checked by hand, the sampling results were put into an Excel spreadsheet for automatic computation, where some errors were found and corrected. In addition, the “most common animal” determined in the field was checked against the laboratory analysis and found to be erroneous in two cases. Specifically at Sites 4 and 10, riffle beetles and snails, respectively, were found in the lab to be most numerous. The first type of error (calculation error) can be avoided by automatic calculation, while the second type (most common animal) is more difficult to avoid.

Figure 18 shows the WAV-1and -2 indices, plotted versus site latitude. The Rush and its three tributaries have been combined into a single plot. This plot shows that the WAV-1 results all lie within a narrow range between 2 and 3. About half the sites are in the good (2.6-3.5) range and half in the fair (2.1-2.5) range. This indicates that, for this dataset, this index does not give a good measurement of stream health. The information incorporated in the calculation of the WAV-1 index is limited due to the order-level grouping of organisms, which does not account for the significant variation of pollution tolerance that may exist for organisms within the same order. For example, *Brachycentrus occidentalis* and *Glossosoma intermedium* caddisflies have very low tolerance values (1 and 0 respectively,) while *Ceratopsyche morosa bifida*, which was abundant at Site 16, has a tolerance value of 6.

WAV-2 does not address this deficiency. However, by virtue of the +1 addition, the WAV-2 index exhibits a wider range of values, as shown in Figure 18. Two sites are in the “excellent” (3.6+) range. As noted above the “most common animal” was incorrectly judged in the field for Sites 4 and 10. Therefore, a correction was made for these sites, with the corrected values shown in the figure. The correction results in a lower value for both sites, in particular Site 10 where snails (a tolerant organism) were found to be the most numerous in the sample.

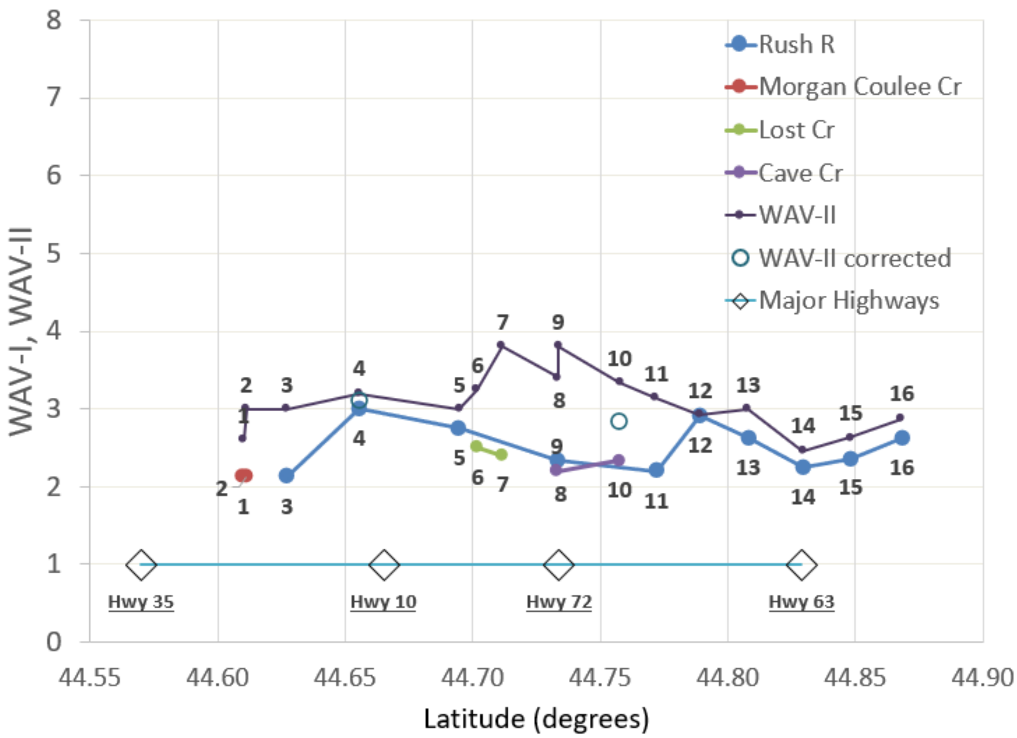

Figure 18. WAV-1 (lower curves) and WAV-2 index vs. latitude.

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To evaluate the utility of the WAV indices as a coarse-level metric, we plotted both WAV-1 and WAV-2 versus HBI. Figure 19 shows this plot for WAV-I. Ideally, the data would lie along a relatively smooth curve, allowing a conversion of the WAV index into the HBI. Further, the data should lie near the target curve indicating the water quality. As seen in the figure, this target curve has a negative slope due to the opposite trending pollution tolerance values (increasing vs. decreasing) for the two indices. Figure 19 shows a large amount of "scatter" in the WAV-1 versus HBI. The very low value of R^2 shows a poor fit by the straight (dashed) line shown on the plot. (Mathematically, R^2 is the square of the correlation coefficient, and measures how well the HBI value predicts the WAV-1 index, or *vice versa*.) In addition, the data are well below the target water quality curve and does not exhibit a negative slope.

As noted above, WAV-2 redefines the tolerance groups and adds the +1 for the most common group. We carried out an identical analysis of WAV-2 versus HBI, and the results are shown in Figure 20. The results are similar to WAV-1. Although the points are moved closer to the target line, there is still a high scatter, and the data cannot be fit to a curve. No downward trend (negative slope) is discernible. However, since a cluster of points (about half) appears to be trending somewhat close to the target line, WAV-2 may be a step in the right direction.

The above indicates that neither WAV-1 nor WAV-2 is a good predictor of HBI (and hence of organic pollution/water quality) for this specific set of data. We emphasize that any conclusions regarding WAV-1 or WAV-2 must be based upon a much larger data set. Since the use of a coarse-level metric offers tremendous benefits, it is worthwhile pursuing this further, with further refinements incorporating as much information as can practically be gathered in the field. Taxonomic skills of volunteers are a critical element of these metrics.

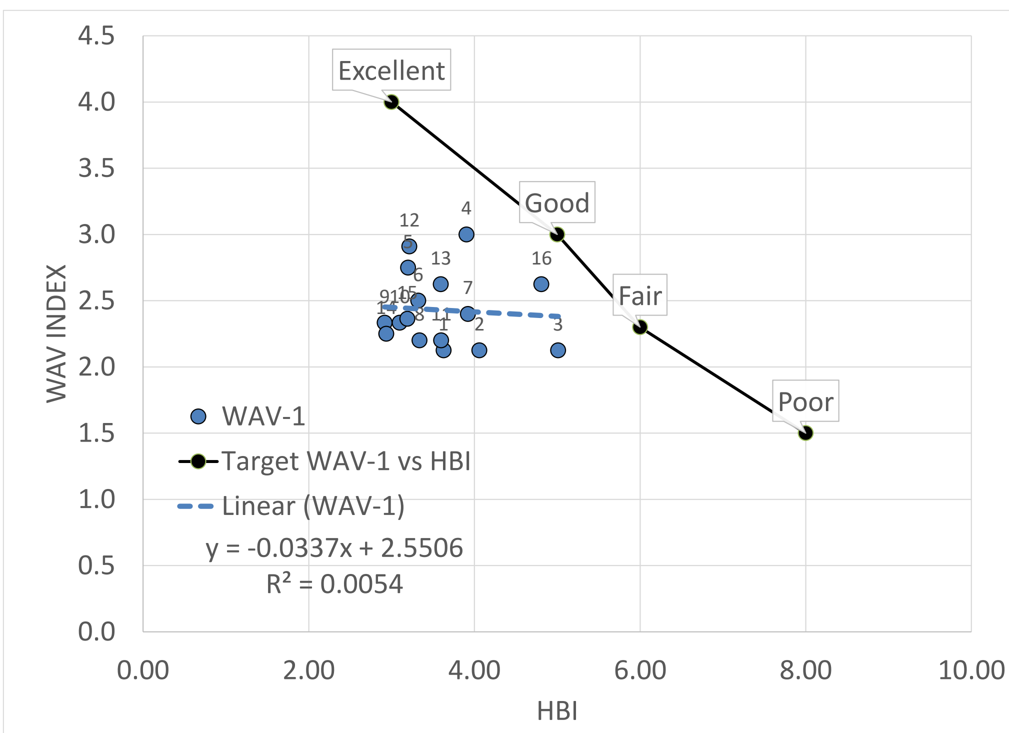

Figure 19. WAV-1 index vs. HBI.

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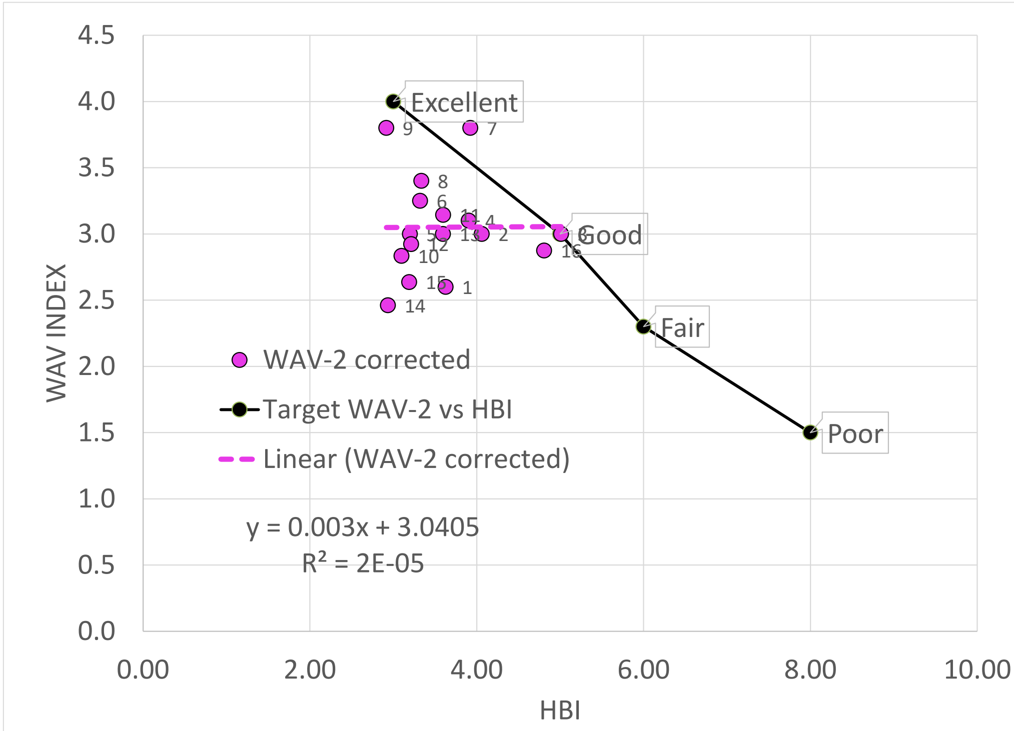

Figure 20. WAV-2 index (corrected) vs. HBI.

HBI Historical Analysis

The results given in the previous sections of this report for the 2023 sampling should be evaluated within the context of a larger dataset which ideally would include historical results over many decades. These results are available on the SWIMS database for most of the 16 sample sites. Six of the 10 Rush River sites and four of the six tributary sites have at least some historical HBI values. These date back as far as 1979 in some cases. However, very few (only one that we could find) have been recorded since 2010.

Figures 21 and 22 show HBI versus site number for the main river and tributaries respectively, plotted separately for clarity, and with historical HBI data added for the sites where it is available. Red points above the curve indicate past HBI values that are higher than current, while points below the curve are values that were lower in the past.

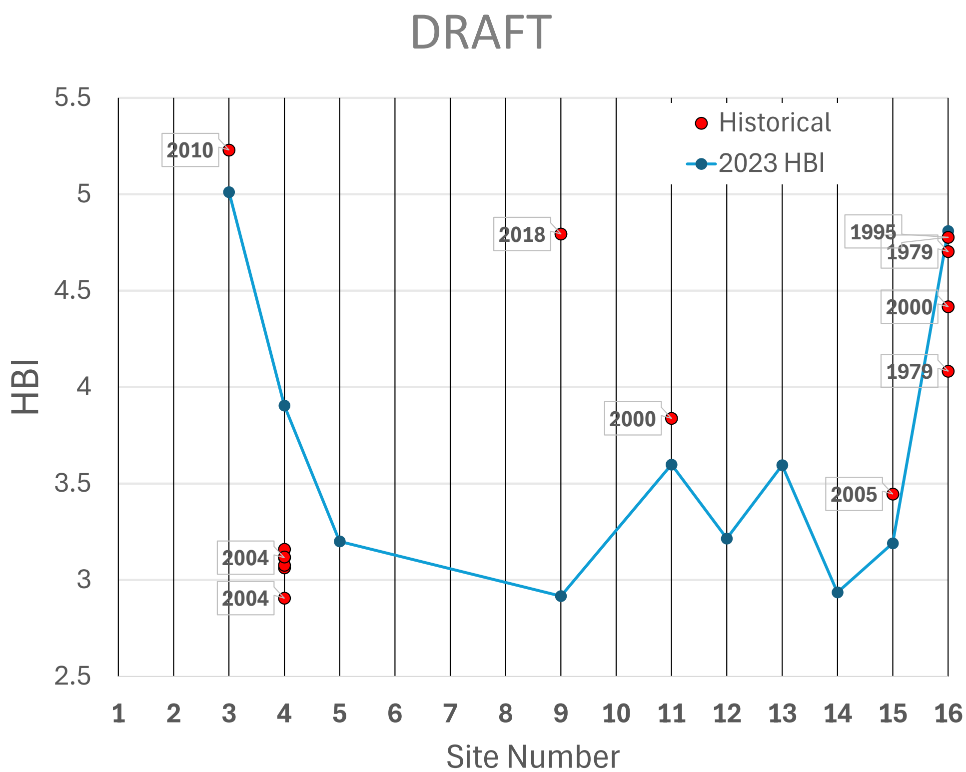

Figure 21. HBI vs. site number for Rush River with historical data.

With the limited amount of historical data, it is impossible to draw any definite conclusions. However, the data are useful in identifying any sites or section of the river where efforts should be concentrated in future monitoring. Where the points are below the curve, it may indicate a trend toward higher organic pollution. One such site on the Rush is Site 4 where a significant increase in HBI was found relative to the 2004 sampling, when six samples yielded an average HBI of 3.07. The current value of 3.90 represents an increase of 27 percent. This sharp increase, as mentioned earlier, is attributed to lower numbers of the *Emphemerella excrucians* mayfly. This site should be monitored closely to determine whether *E. excrucians* is in fact declining, and if it is, to investigate the cause.

A second site with historical HBI points below the 2023 curve is Site 16. Currently, this site has a relatively high HBI of 4.81. This site is unique, with a taxa composition that includes a high percentage (>50%) of caddisflies, predominantly *Ceratopsyche morosa bifida* and a low percentage (<10%) of mayflies. *C. morosa bifida* is a net-spinning filter feeder, known to be abundant in waters below impoundments where algae is found in the water column (Michael Miller, Kurt Schmude, Personal Communications, 2024.)

Historical data for Site 16 suggest that the HBI may be trending upward, indicating a possible increase in organic pollution. We note that historical data is a mixture of fall, spring, and summer samples. This site also has a much higher HBI than the nearest site downstream. All the above factors point to the need for more data.

The historical results for the tributaries (Fig. 22) show a similar situation. Historical HBI datapoints lie both above and below the 2023 points. There is no clear trend, and more sampling is needed to draw conclusions.

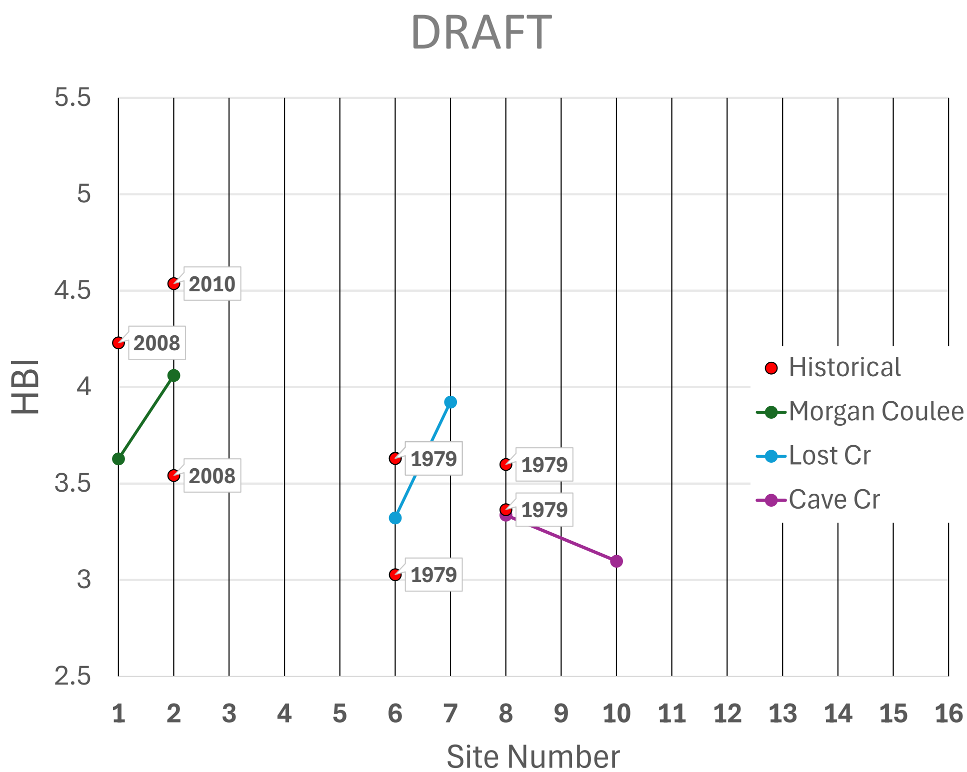

Figure 22. HBI vs. site number with historical data for the tributaries.

The current HBI values, combined with the historical data, point to the need to concentrate on the lower (below Highway 10) and upper section (above Highway 29) of the Rush River. These two sections appear to have the highest, and possibly increasing, levels of organic pollution.

Conclusion

Although it remains a high-quality fishery, the Rush River is not immune from the many stressors facing our trout streams. These streams and the surrounding landscape are sensitive ecosystems that depend upon a complex food web, with macroinvertebrates being a critical element for energy transfer from organic matter to higher trophic levels. These organisms have a range of tolerance to low levels of dissolved oxygen resulting from organic pollution. Thus, the composition of the macroinvertebrate population provides valuable data on stream health. The Rush River Macroinvertebrate Monitoring Project is a volunteer-based effort to ensure that macroinvertebrate data will be available into the future over most of the Rush River system, including important tributaries. The goals for the initial 2023 sampling were ambitious; 16 sampling sites were selected to cover a large portion of the river, and these should form a foundation for future work.

This report represents a "snapshot" in time. It presents a large volume of data, which must be verified, integrated with a larger database, and updated continuously to form a picture of the river's evolving health. Thus, there is much more work to be done in the near future.

Note on the SWIMS Database

The metrics presented in this report were computed by Dr. Kurt Schmude and the author, after it was found that the metrics provided by SWIMS were erroneous. It is our understanding that the SWIMS team is working to correct these computations. The author will continue to work to ensure that the metrics reported here are in adequate agreement with the values on SWIMS when corrections are complete. Due to the many taxa and tolerance values involved, there may be small discrepancies. This report will be updated if necessary.

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References

Engel, MP, Michalek, WJ. 2002. Rush River Watershed Comprehensive Surface Water Resource Report, St. Croix, Pierce and Pepin Counties, Wisconsin Department of Natural Resources.

Hilsenhoff, WL. 1982. Using a biotic index to evaluate water quality in streams. Technical Bulletin (132) 1-22, Wisconsin Department of Natural Resources, Madison, WI.

Hilsenhoff, WL. 1987. An improved biotic index of organic stream pollution. *The Great Lakes Entomologist*, 20(1) 31-39.

Hilsenhoff, WL. 1988. Rapid field assessment of organic pollution with a family-level biotic index. *Journal of the North American Benthological Society*, 7:65-68.

Houghton, DC, DeWalt, RE. 2023. The caddis aren't alright: modeling Trichoptera richness in streams of the northcentral United States reveals substantial species losses. *In Frontiers in Ecology and Evolution* (Vol. 11). Frontiers Media SA.

Lillie, RA, Szczytko, SW, Miller, MA. 2003. Macroinvertebrate Data Interpretation Guidance Manual. Wisconsin Department of Natural Resources, Bureau of Integrated Science Services. Madison, WI. PUB-SS-965-2003.

Nelson, CA. 2019. Lower Rush River: Present Health and A Call to Action.

O'Harrow Jr., R. (2022, September 19). The World's Oldest Winged Insect Is in Trouble. How Frightened Should We Be? *The Washington Post*. https//www.washingtonpost.com/magazine/2022/09/19/mayfly-decline/

Schmude, KL, Hilsenhoff, WL. 1986. Biology. Ecology, Larval Taxonomy, and Distribution of Hydropsychidae (Trichoptera) in Wisconsin. *The Great Lakes Entomologist*, 19 (3) 123-145.

WDNR 2017. Guidelines for the Standard Collection of Macroinvertebrate Samples from Wadeable Streams v2.0. Wisconsin Dept. of Natural Resources, Madison, WI.

Weigel, B.M. 2003. Development of stream macroinvertebrate models that predict watershed and local stressors in Wisconsin. *Journal of the North American Benthological Society*, 22(1):123-142.

Yallaly, K. 2021. Fisheries survey report for Rush River, Pierce and St. Croix County, Wisconsin 2021, waterbody identification code 2440300. WDNR Fisheries Biologist, Baldwin, WI.

APPENDIX A– LIST OF TAXA BY SITE (FROM K. SCHMUDE SAMPLING RESULTS SPREADSHEET)

Mayflies, Stoneflies, Caddisflies, Hellgrammites, Beetles, and True Bugs

	MC Cr	MC Cr	Rush R	Rush R	Rush R	Lost Cr	Lost Cr	Cave Cr	Cave Cr	Rush R	Rush R	Rush R	Rush R	Rush R	Rush R	Rush R
Sampling Date	9/21/23	9/21/23	9/29/23	9/29/23	9/29/23	10/12/23	10/12/23	10/18/23	10/18/23	10/12/23	10/18/23	10/2/23	10/2/23	10/2/23	10/2/23	10/2/23
Station Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**9**	**11**	**12**	**13**	**14**	**15**	**16**
EPHEMEROPTERA (mayflies)																
Acentrella turbida			5		6											
Baetis brunneicolor	17	38	3	2		1					2			3	1	
Baetis flavistriga group		3	70	5	34		1	1		3	4		2		2	
Baetis tricaudatus	2	16	1	1	2	34	4	67	61	37	23	1	3	10	7	
Iswaeon anoka				5		3				2	2	1	2	1		
Ephemerella excrucians (immature)			8	11	26	8				1				1	1	
Teloganopsis deficiens			1	7	44	13				120	84	122	114	128	91	22
Maccaffertium mediopunctatum			2							1				1		
Maccaffertium vicarium					4	4		2		7	1	2			1	1
Tricorythodes				1		1								1	1	
PLECOPTERA (stoneflies)																
Paragnetina media				1												
Isoperla (immature)				3	1							1	3	2		2
Isoperla signata						25	2			1						
Pteronarcys						1										
Taeniopteryx			1													
TRICHOPTERA (caddisflies)																
Brachycentrus occidentalis	5	4		1		26	19	7								
Glossosoma intermedium	24	4							5						2	
Protoptila			2	4	22					1		1		1		
Helicopsyche borealis								1		2		2				6
Ceratopsyche alhedra	1		1	35	12	18	21	5		15	14		1	5	66	8
Ceratopsyche bronta	1		2	12	9	1		1		3	14		2		1	13
Ceratopsyche morosa bifida				11	9							2	3	1	6	77
Ceratopsyche slossonae	23	21				10	17	54	28		5	1	10	10	8	10
Cheumatopsyche	1	6	2	5	8	5	5	9		13	2		5	15	8	13
Hydropsyche betteni	1			1							4					
Hydroptila							2	1			2			1		1
Lepidostoma		4										1				
Ceraclea																1
Oecetis															1	
Oecetis avara						5		1		4			2			
Limnephilidae (immature)		3														
Hesperophylax designatus		1							7							
Limnephilus indivisus	1															
Chimarra aterrima										5	2					
Psychomyia flavida				1	4						1				2	2
MEGALOPTERA																
Nigronia serricornis												1				
COLEOPTERA (beetles)																
Helichus striatus	2	5		1			1									
Optioservus fastiditus	65	10	3	134	62	41	34	2	3	23	31	54	42	25	57	36
Stenelmis (larvae)					1							1				
HEMIPTERA (true bugs)																
Belostoma flumineum	1															
Sigara trilineata			25													

Midges, Black Flies, and Crane Flies

	MC Cr	MC Cr	Rush R	Rush R	Rush R	Lost Cr	Lost Cr	Cave Cr	Cave Cr	Rush R	Rush R	Rush R	Rush R	Rush R	Rush R	Rush R
Sampling Date	9/21/23	9/21/23	9/29/23	9/29/23	9/29/23	10/12/23	10/12/23	10/18/23	10/18/23	10/12/23	10/18/23	10/2/23	10/2/23	10/2/23	10/2/23	10/2/23
Station Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**9**	**11**	**12**	**13**	**14**	**15**	**16**
DIPTERA (flies)																
Atherix variegata				4								2	1	2	1	1
Ceratopogoninae		5							1	1						
Chaetocladius		1														
Chironomus			7													
Cladotanytarsus			5	4												
Conchapelopia		1	1			2	3	1		4			2	3	1	1
Corynoneura			1			2		1					2			1
Cricotopus			1				1				2	1				
Cricotopus bicinctus						1		3		1						2
Cricotopus trifascia					4			1		1						
Diamesa						2	1									
Dicrotendipes			2													
Eukiefferiella (too immature)				2									5			
Eukiefferiella (pupa)							1									
Eukiefferiella brehmi group						7					4			1		
Eukiefferiella claripennis												1				
Eukiefferiella devonica group				2		1	6				9	5	2		1	2
Eukiefferiella gracei group					1											
Limnophyes		1														
Micropsectra			1													
Microtendipes pedellus group			3		4		1	1		1		3	12	1	1	9
Nanocladius														1		
Natarsia													2			
Nilothauma												2				
Orthocladius			3			3	6	15	1	2	2					12
Pagastia						1	5	14								
Paracladopelma			1													
Parachaetocladius		7	1													
Paramerina		1														
Parametriocnemus	1	1	1					1	1		1			1	1	5
Paratanytarsus							1							1	1	
Paratanytarsus longistilus	1						4		1	1	2	3	2			
Paratendipes								1	1				2	1		
Polypedilum						6		1								9
Polypedilum scalaenum group													3			
Potthastia longimanus group														1		
Rheotanytarsus	1		2	2		1				1	8	7	2			2
Stictochironomus			19													
Sublettea coffmani				1				1			3	6	3	1	1	
Synorthocladius semivirans	1															
Tanytarsus		10	6			2	4	1	4	1	1	3	1			
Thienemanniella					1	1				1	1		1		2	4
Tvetenia paucunca	6			4	3	2	2			3	8	3	8	3	3	1
Tvetenia tshernovskii				1				1		2	2	4	3	1	4	1
Dixa	1															
Hemerodromia							1			1	1	3	2	1		2
Neoplasta	2						1	1	1							
Limnophora																1
Pericoma									3							
Simulium (damaged)					1											
Simulium tuberosum group	3	6		1				2	3		2		4	1		
Simulium vittatum group 4	1	8	4	2		6	5	1			1		1		3	
Chrysops		1														
Antocha					1	1	8	1		3	4	4	2		2	10
Dicranomyia									1							
Dicranota	4	5				4		1	3							
Eloeophila (=Limnophila)		3														
Gonomyia/Idiocera/Ellipteroides	1															
Pilaria			1													1
Tipula	1												2	3	1	

Scuds, Mites, Worms, Leeches, Snails, and Clams

	MC Cr	MC Cr	Rush R	Rush R	Rush R	Lost Cr	Lost Cr	Cave Cr	Cave Cr	Rush R	Rush R	Rush R	Rush R	Rush R	Rush R	Rush R
Sampling Date	9/21/23	9/21/23	9/29/23	9/29/23	9/29/23	10/12/23	10/12/23	10/18/23	10/18/23	10/12/23	10/18/23	10/2/23	10/2/23	10/2/23	10/2/23	10/2/23
Station Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**9**	**11**	**12**	**13**	**14**	**15**	**16**
AMPHIPODA (scuds)																
Gammarus pseudolimnaeus	104	15	13	6	6	1		3	27	3	2		1	5	1	1
ISOPODA (sowbugs)																
Caecidotea														1		
HYDRADCHNIDA (mites)																
Aturus		1						1					3	3	1	
Hygrobates		14	3	14		2	26	5	2	2	1	20	5	7	2	
Lebertia		5					3	1	4	1	1		1			
Sperchon	3	9	1	7	1	10	12	13	5	2	7	3	4	12	3	
Torrenticola									1		1	1	1		1	
CNIDARIA																
Hydra		2														
OLIGOCHAETA (worms)																
Enchytraeidae		7				2	1		2	1						
Lumbriculidae									2							
Tubificinae immature without hairs		4	23				2		13	2	2					
Tubificinae immature with hairs			5						6							
Ilyodrilus templetoni			1													
Limnodrilus hoffmeisteri									1							
Nais behningi			2	1				1		1	1	4	2	3		
Nais bretscheri	12	1	2	1	2	3	37	40	2	5	4	5	2			
Nais communis		2	12				1	1	10							
Nais pardalis				2					1	3	1					1
Nais simplex								6	1						1	
Ophidonais serpentina	3													1		
Pristina leidyi			2													
HIRUDINEA (leeches)																
Erpobdella parva					1											
Glossiphonia elegans						2								1		
TURBELLARIA (flatworms)	3			1		5	1	6		3	4	4	1	16	6	
NEMERTEA - Prostoma (proboscis worm)				4												
GASTROPODA (snails)																
Stagnicola									5							
Physa	5	48	2		1		9	1	69	1	1	1		13	16	2
Gyraulus							2	1	1					1	1	
BIVALVIA (fingernail clams)																
Pisidium			1					2	8							
Sphaerium												4	1		1	

APPENDIX B– LIST OF TAXA BY TOTAL COUINT

rank	number	taxon			
1	746	Teloganopsis deficiens	76	6	Gyraulus
2	622	Optioservus fastiditus	77	6	Sphaerium
3	269	Baetis tricaudatus	78	5	Capniidae (too immature)
4	202	Ceratopsyche alhedra	79	5	Lepidostoma
5	197	Ceratopsyche slossonae	80	5	Cricotopus
6	188	Gammarus pseudolimnaeus	81	5	Paratendipes
7	169	Physa	82	5	Neoplasta
8	125	Baetis flavistriga group	83	5	Torrenticola
9	116	Nais bretscheri	84	5	Stagnicola
10	109	Ceratopsyche morosa bifida	85	4	Maccaffertium mediopunctatum
11	103	Hygrobates	86	4	Tricorythodes
12	97	Cheumatopsyche	87	4	Ophidonais serpentina
13	92	Sperchon	88	4	NEMERTEA - Prostoma (proboscis worm)
14	67	Baetis brunneicolor	89	3	Maccaffertium (too immature)
15	62	Brachycentrus occidentalis	90	3	Limnephilidae (too immature)
16	59	Ceratopsyche bronta	91	3	Diamesa
17	56	Ephemerella (too immature, prob. excrucians	92	3	Paratanytarsus
18	50	TURBELLARIA (flatworms)	93	3	Polypedilum scalaenum group
19	46	Tvetenia paucunca	94	3	Pericoma
20	46	Tubificinae immature without hairs	95	3	Eloeophila (=Limnophila)
21	44	Orthocladius	96	3	Glossiphonia elegans
22	36	Microtendipes pedellus group	97	2	Stenelmis (larvae)
23	36	Antocha	98	2	Dicrotendipes
24	35	Glossosoma intermedium	99	2	Natarsia
25	33	Tanytarsus	100	2	Nilothauma
26	32	Simulium vittatum group 4 tolv 4 or 7	101	2	Pilaria
27	31	Protoptila	102	2	Hydra
28	28	Isoperla signata	103	2	Lumbriculidae
29	28	Eukiefferiella devonica group	104	2	Pristina leidyi
30	26	Rheotanytarsus	105	1	Paragnetina media
31	26	Nais communis	106	1	Pteronarcys
32	25	Sigara trilineata	107	1	Taeniopteryx
33	22	Maccaffertium vicarium	108	1	Ceraclea
34	22	Simulium tuberosum group	109	1	Oecetis
35	20	Pagastia	110	1	Limnephilus indivisus
36	19	Conchapelopia	111	1	Nigronia serricornis
37	19	Stictochironomus	112	1	Belostoma flumineum
38	19	Tvetenia tshernovskii	113	1	Chaetocladius
39	17	Dicranota	114	1	Eukiefferiella (pupa)
40	16	Iswaeon anoka	115	1	Eukiefferiella claripennis
41	16	Polypedilum	116	1	Eukiefferiella gracei group
42	16	Sublettea coffmani	117	1	Limnophyes
43	16	Lebertia	118	1	Micropsectra
44	15	Nais behningi	119	1	Nanocladius
45	14	Paratanytarsus longistilus (10?)	120	1	Paracladopelma
46	13	Parametriocnemus	121	1	Paramerina
47	13	Enchytraeidae	122	1	Potthastia longimanus group
48	12	Isoperla (too immature)	123	1	Synorthocladius semivirans
49	12	Oecetis avara	124	1	Dixa
50	12	Eukiefferiella brehmi group	125	1	Limnophora
51	11	Acentrella turbida	126	1	Simulium (damaged)
52	11	Helicopsyche borealis	127	1	Chrysops
53	11	Atherix variegata	128	1	Dicranomyia
54	11	Thienemanniella	129	1	Gonomyia/Idiocera/Ellipteroides
55	11	Hemerodromia	130	1	Caecidotea
56	11	Tubificinae immature with hairs	131	1	Ilyodrilus templetoni
57	11	Pisidium	132	1	Limnodrilus hoffmeisteri
58	10	Psychomyia flavida	133	1	Erpobdella parva
59	9	Helichus striatus			
60	9	Cladotanytarsus			
61	9	Aturus			
62	8	Hesperophylax designatus			
63	8	Parachaetocladius			
64	8	Nais pardalis			
65	8	Nais simplex			

APPENDIX C – PHOTOS OF DISCHARGE AT SITE 4

September 29, 2023. Downstream of Discharge at Site 3 (385th St Bridge.)

September 29, 2023. **October 13, 2023**

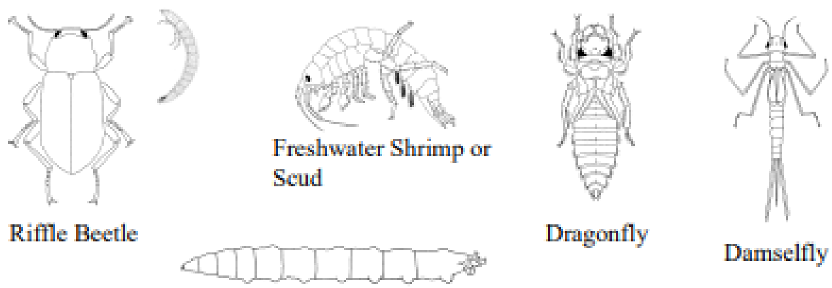
Point of Discharge at Site 4 (CTH A South of Hwy 10)

APPENDIX D – WAV-2 DRAFT INDEX RECORDING FORM

Group 1. These animals are most sensitive to pollution. Circle the name of each animal found.

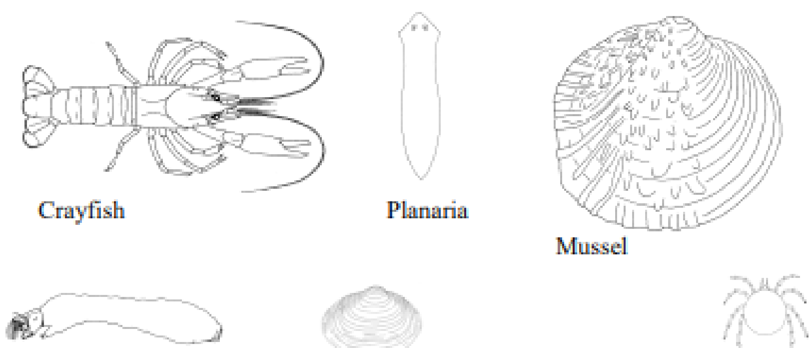
No. of Group 1 animals found: ☐

Group 2. These animals are less sensitive to pollution than Group 1. Circle the name of each animal found.



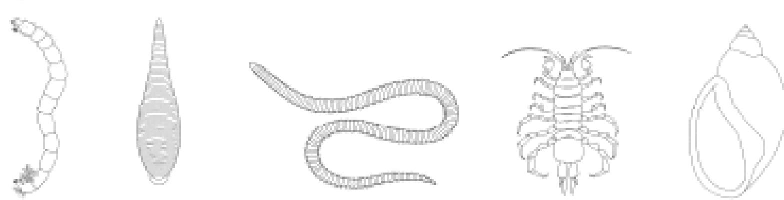
No. of Group 2 animals found: ☐

Group 3. These animals are more tolerant of pollution than Groups 1 or 2. Circle the name of each animal found.



No. of Group 3 animals found: ☐

Group 4. These animals are most tolerant of pollution. Circle the name of each animal found.



No. of Group 4 animals found: ☐

Recording Form for the Citizen Monitoring Biotic Index

Name: ____ Date: ______________ Watershed and Stream Names: _______________________________ Time: ______________ Location: ____ Site: _________________ (County, Township, Range, Section, Road, Intersection, Other)

At this point, you should have collected a wide variety of aquatic macroinvertebrates from your three sites. You will now categorize your sample, using the *Key to Macroinvertebrate Life in the River* to help you identify the macroinvertebrates found. **The number of animals found is not important; rather, the variety of types of macroinvertebrates and their tolerance to pollution tells us the biotic index score**. Before you begin, check off the habitats from which you collected your sample (see right).

- ☐ Riffles
- ☐ Undercut banks
- ☐ Snag areas, tree roots, submerged logs
- ☐ Leaf packs

1. You should have removed large debris (e.g. leaves, rocks, sticks) from your sample and placed this material in a separate basin (after removing macroinvertebrates from it).

2. Check the basin with the debris to see if any aquatic macroinvertebrates crawled out. Add these animals to your sample.

3. Fill the ice cube tray half-full with water.

4. Using plastic spoons or tweezers, (be careful not to kill the critters – ideally, you want to put them back in their habitat after you're finished) sort out the macroinvertebrates and place ones that look alike together in their own ice cube tray compartments. Sorting and placing similar looking macroinvertebrates together will help insure that you find all varieties of species in the sample.

5. Refer to the *Key to Macroinvertebrate Life in the River* and the *Citizen Monitoring Biotic Index* to identify the aquatic macroinvertebrates:

A. On the back of this page, circle the animals on the index that match those found in your sample.

B. Count the number of types of animals that are circled in each group and write that number in the box provided. Do not count individual animals in your sample. Only count the number of types of animals circled in each group.

C. Enter each boxed number in the first space provided.

D. Decide which animal is the most common; add 1 for that animal's group in the second space provided (shaded area). Add zero for all other groups.

E. Multiply the entered sum by the group value.

F. Total the number of animals circled (a).

G. Total the calculated values for all groups (b).

H. Divide the total values by the total number of types of animals that were found: **TOTAL VALUES (b) / TOTAL ANIMALS (a)**.

I. Record this number as the Index score. SHOW ALL MATH (Use space below to do your math computations)

		+1 for group with the most common animal (+0 for the other three groups)				
No. of animals circled from group 1	_____	+ ____	=	____	x 4 =	_____
No. of animals circled from group 2	_____	+ ____	=	____	x 3 =	_____
No. of animals circled from group 3	_____	+ ____	=	____	x 2 =	_____
No. of animals circled from group 4	_____	+ ____	=	____	x 1 =	_____
	Total animals (a)					**Total values (b)**

(b) total values _____ ÷
(a) total animals _____ =

Index score:

How healthy is the stream?

Excellent	3.6+
Good	2.6 - 3.5
Fair	2.1 - 2.5
Poor	1.0 - 2.0

*Call your local Monitoring Coordinator if you have questions about sampling or determining the Biotic Index Score. **Return form to:***

APPENDIX E – WAV-1 AND WAV-2 COMPUTATIONS

Site	Description	Group 1	Group 2	Group 3	Group 4	total spec.	total values	WAV-1	rating
1	Morgan Coulee Cr - 385th St	0	3	3	2	8	17	2.1	Fair
2	Morgan Coulee Cr - 200th Ave	0	3	3	2	8	17	2.1	Fair
3	Rush River - 385th St	0	3	3	2	8	17	2.1	Fair
4	Rush River - 2000m south of HWY 10	2	4	2	0	8	24	3.0	Good
5	Rush River - 450th Ave	2	3	2	1	8	22	2.8	Good
6	Lost Creek - 465th Ave	1	4	1	2	8	20	2.5	Fair
7	Lost Creek - 450th St	0	3	1	1	5	12	2.4	Fair
8	Cave Creek - Hwy 72	0	2	2	1	5	11	2.2	Fair
9	Rush River - Hwy 72 - NRSA Site	0	3	2	1	6	14	2.3	Fair
10	Cave Creek - 610th Ave	0	3	2	1	6	14	2.3	Fair
11	Rush River - Hwy N El Paso	0	2	2	1	5	11	2.2	Fair
12	Rush River - Stonehammer	3	5	2	1	11	32	2.9	Good
13	Rush River - Wonderland	0	5	3	0	8	21	2.6	Good
14	Rush River - Hwy 63 Martell	1	4	4	3	12	27	2.3	Fair
15	Rush River - Hwy 29	1	4	4	2	11	26	2.4	Fair
16	Rush River - CTH Y	1	4	2	1	8	21	2.6	Good

Site	Description	Group 1	+1	Group 2	+1	Group 3	+1	Group 4	+1	total spec.	total values	WAV-2	rating
1	Morgan Coulee Cr - 385th St	2	0	2	1	3	0	3	0	10	26	2.6	Good
2	Morgan Coulee Cr - 200th Ave	2	1	2	0	2	0	2	0	8	24	3.0	Good
3	Rush River - 385th St	2	1	2	0	2	0	2	0	8	24	3.0	Good
4	Rush River - 2000m south of HWY 10	3	1	3	0	3	0	1	0	10	32	3.2	Good
5	Rush River - 450th Ave	3	1	2	0	3	0	2	0	10	30	3.0	Good
6	Lost Creek - 465th Ave	3	1	2	0	1	0	2	0	8	26	3.3	Good
7	Lost Creek - 450th St	2	1	2	0	0	0	1	0	5	19	3.8	Excellent
8	Cave Creek - Hwy 72	2	1	1	0	0	0	2	0	5	17	3.4	Good
9	Rush River - Hwy 72 - NRSA Site	2	1	2	0	0	0	1	0	5	19	3.8	Excellent
10	Cave Creek - 610th Ave	2	1	2	0	0	0	2	0	6	20	3.3	Good
11	Rush River - Hwy N El Paso	2	1	2	0	1	0	2	0	7	22	3.1	Good
12	Rush River - Stonehammer	4	1	2	0	5	0	2	0	13	38	2.9	Good
13	Rush River - Wonderland	2	1	3	0	4	0	1	0	10	30	3.0	Good
14	Rush River - Hwy 63 Martell	2	1	3	0	3	0	5	0	13	32	2.5	Fair
15	Rush River - Hwy 29	2	1	3	0	2	0	4	0	11	29	2.6	Fair
16	Rush River - CTH Y	2	1	2	0	1	0	3	0	8	23	2.9	Good

corrected

Site	Description	Group 1	+1	Group 2	+1	Group 3	+1	Group 4	+1	total spec.	total values	WAV-2	rating
4	Rush River - 2000m south of HWY 10	3	0	3	1	3	0	1	0	10	31	3.1	Good
10	Cave Creek - 610th Ave	2	0	2	0	0	0	2	1	6	17	2.8	Good