

# SHORT-TERM TRENDS AMONG BENTHIC MACROINVERTEBRATE ASSEMBLAGES IN THE WOOD RIVER BASIN

AN ASSESSMENT OF SOME IMPORTANT ECOLOGICAL METRICS INDICATIVE OF WATER  
QUALITY FROM 2022-2024, INCLUSIVE

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**COVER PHOTO.** The ephemereid mayfly, *Ephemerella excrucians*, is a common mayfly of the streams in the Wood River basin. This mayfly species and its close relatives are commonly referred to as ‘pale morning duns.’ Image copyright B. Marshall, Manhattan, MT, [Rivercontinuum@gmail.com](mailto:Rivercontinuum@gmail.com).

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## EXECUTIVE SUMMARY

Wood River Land Trust (WRLT) began monitoring benthic macroinvertebrates in 2022 and continued through 2024. This report examines the changes in space and time that have occurred among these monitoring sites.

The approach depended on the use of six ecological metrics used to summarize the ecological conditions at six sites in March/April of each year. Representative sampling was assured through the collection of five individual statistical replicates from each site during each sampling period. Although this report focuses on a subset of metrics that summarize ecological changes and explain why changes occur, they are only a subset of the metrics deliver to WRLT.

## CHANGES AT INDIVIDUAL SITES OVER TIME

The most important temporal trends at each site are described in greater detail within the report. Here they are presented as a bulleted list.

### BLWMAG:

- Declines in Total taxa richness and EPT richness in 2023 and 2024. (negative)
- These declines were offset by a gradual increase in EPT abundance. (positive)
- Reductions in chironomid midge and non-insect abundance. (positive)

### BWSTANT:

- Total Taxa Richness and EPT richness reduced in 2023. (negative)
- This was off-set by increases in EPT abundance. (positive)
- Declines in the abundance of midges and non-insects. (positive)
- The HBI described a marked and statistically significant improvement in 2024 compared to prior years. (positive)

### BWHAILEY

- There was a reduction in Total taxa richness in 2023 (negative), but it rebounded in 2024. (positive)
- EPT Abundance (>80%) was abnormally high in 2023. Usually, high EPT Abundance is reflective of improved conditions. However, sometimes abnormally high EPT Abundance can indicate a location with frequent intermittent disturbances (e.g., point sources). AT BWHAILEY, this was accompanied by a reduction in Richness (Total and EPT) and thus may represent recolonization after an intermittent disturbance (see text). EPT abundances were more normal in 2022 and 2024—so this did not seem to be part of trend.
- The HBI was best in 2023 because it reflects the increase in EPT abundance, but not the loss in richness.

## EFORK

- In 2023, average EPT richness (9.6) was close to the threshold of concern (<9.0) but was not statistically significant from observations in 2022 or 2023.
- EPT abundances appeared to increase incrementally over time.
- HBI generally declined (improvement) over time, responding to an increase in sensitive species, primarily EPT's.

## WARMSP

- Increases in Total taxa richness and EPT richness suggested that conditions improved over time. (positive)
- The site started the monitoring period with EPT richness (8.0), which was below the threshold for concern (< 9.0). (negative)
- In 2023 and 2024 increases in EPT Abundance were accompanied by increases in EPT richness. This was examined and it appeared that an increase in the richness and density of many EPT taxa were involved (supplementary figure provided). (Very positive)
- HBI integrated all these changes and demonstrated marked improvements in 2023 and 2024 compared to 2022. (positive)

## BWNSRA

- Average values of all metrics were consistently reflective of good water quality and habitat quality.
- However, 1 sample in 2023 had abnormally high midge abundance (~80%).
- Two samples in 2024 had abnormally high non-insect abundances (>20%) but not exceptionally so.
- The site expressed a consistently low ("excellent") average HBI level. (positive)

## DIFFERENCES AMONG SITES EACH YEAR

### Total taxa richness

- No significant differences among sites in 2022, 2023, or 2024.
- Total Richness was lowest at WARMSP in 2022.
- None of the sites stood out as exceptional in terms of average Total Richness in 2023, but BWNSRA had several exceptionally high individual observations.
- Total Richness was lower on average in 2023 than the other years
- Lowest Taxa Richness in 2024 occurred at BLWMAG

### EPT richness

- In 2022, the only statistically significant difference in EPT richness occurred between the lowest site (WARMSP, 9 EPT taxa) and the site with the highest average EPT richness (BWNSRA, 15 EPT taxa).
- In 2023 there was a clear gradient in EPT richness, which increased farther up the watershed and decreased lower in the watershed. BLWMAG had an average EPT < 5.0.
- In 2024, the apparent gradient was similar to that observed in 2023, with very low richness at BLWMAG (5.0 EPT taxa) and the greatest occurring at EFORK.

### EPT Relative Abundance

- In 2022, EPT Abundance was abnormally low at BLWMAG and BWSTANT. (negative)
- In 2022, EPT Abundance was greatest at BWHAILEY and BWNSRA. (positive)
- In 2023, EPT Abundance was abnormally low at BLWMAG and BWSTANT. (negative)
- In 2023, EPT Abundance was greatest at BWHAILEY and BWNSRA. (positive)
- In 2023, EPT abundances at BLWMAG, BWSTANT and EFORK were statistically significantly reduced compared to BWHAILEY
- In 2024, EPT Abundance was lowest at BWHAILEY, BLWMAG, and EFORK.
- The most notable spatial change in EPT Abundance was that BLWMAG and BWSTANT consistently had fewer EPT's than most other sites.

### Chironomid Midge Abundance

- In 2022, BLWMAG and WARMSP stood out for abnormally high chironomid midge abundance.
- In 2023, average midge abundances were ideal (< 20 %) across all sites.
- In 2024, slightly elevated midge abundances were observed at BLWMAG, BWSTANT, and BWHAILEY
- The lack of clear longitudinal trends in midge abundances indicated that the annual differences at the sites were sensitive to ephemeral inter-annual influences rather than persistent geographic influences—interactions notwithstanding.

### Non-Insect Abundance

- Abnormally high non-insect abundances occurred at BLWMAG and WARMSP in 2022.
- Non-insect abundances were normal in 2023.
- Abnormally high non-insect abundances were observed at BLWMAG, BWSTANT, and BWHAILEY in 2024.

### Hilsenhoff Biotic Index (HBI)

- HBI integrated all the offsetting changes in EPT abundance, midge abundance, and non-insect abundance into one measure reflective of habitat and water quality.
- HBI did not detect changes in Total taxa richness or EPT richness .
- HBI generally indicated that sites higher in the watershed had better water quality and that sites lower in the watershed had somewhat worse water quality.

### Summary of Longitudinal Changes.

- The use of tentative thresholds allowed us to rate each site by the frequency of deviation from expectations.
- The results indicated that BLWMAG was most prone to ecological perturbations (10/15)
- BWSTANT was also very prone to perturbation (8/15).
- EFORK and BWNSRA did not seem prone to perturbation (0/15).

## CONCLUSIONS

- Some limitations to the study methods to improve the design are briefly discussed.
- Changes at individual sites over time were difficult to describe because:
  - Short monitoring period
  - Wide variation in observations
- Spatial Changes each year indicated some sites were more prone to ecological perturbations than others.
- BLWMAG and BWSTANT were the sites more susceptible to perturbation and might become priorities for restoration in the future.
- BWNSRA and EFORK were the sites with no observed deviations in expectations and could become focal points for conservation efforts.
- Future efforts could include site-specific screening criteria, so that we can flag data that deviates from baseline allowing WRLT to disseminate and assimilate the information more rapidly each year.



## INTRODUCTION

Wood River Land Trust began collecting quantitative macroinvertebrate samples from an assortment of 6 sites in the Wood River Basin in the spring of 2022. The monitoring program is ongoing, and the goal is to be able to detect change among sites and over time. This report is a brief summary of the data in the collected from the 2022-2024 sampling periods.

## THE IMPORTANCE OF AQUATIC INSECTS

Donors and community partners of the Wood River Land Trust may wonder why sampling macroinvertebrates is important for watershed management. The short answer is that comprehensive watershed management strategies need objective data to assess ecological changes. In short, you cannot tell how the resource is changing without first defining the starting conditions. The structure of macroinvertebrate communities integrates the collective effects of chemical, physical and biological changes (as well as the interactions within and among these aspects of ecosystem integrity) occurring in any aquatic ecosystem. The longer answer is more nuanced.

Invertebrates are the most important animals in the world. Without them, the processes of pollination, detrital breakdown, and nutrient cycling would stop (or become so slow as to be effectively stopped). This would be so detrimental to life on earth that higher vertebrate life, including humans and fish, would cease to exist in about three years. And yet, if humans were to disappear today, only a tiny portion of co-dependent species would be extirpated.

Invertebrates, and insects in particular, comprise the greatest number of animal species. There are only about 43,000 vertebrate species (including fish, mammals, birds, amphibians, reptiles, and others); whereas there are about 1,000,000 invertebrates known to science. At the rate of species discovery, the true number of insect species (a subset of invertebrate species) has been estimated to be as high as 30 million species. Studies have shown that ecosystems supporting high species diversity have greater productivity, and resilience—while being better able to support ecosystem services—such as fishing & recreation (e.g., Wilson 1988, 1992).

The skeptic might diminish the significance of insects because their individual sizes are relatively small compared to a whale, an elephant or a person. However, they are incredibly abundant. They are so abundant that if you weighed all the animals on earth—including all the fish, whales, people and everything else—then over 90% of the mass would be insects (e.g., Wilson 1988, 1992).

Insects are incredibly diverse and abundant. Insects play critical roles at the base of most terrestrial and freshwater ecosystems. They comprise most of the animal species on earth, with beetles alone representing more species than all other non-insect species combined! You cannot consider the function or health of any ecosystem (including marine systems) without accounting for the roles of invertebrates.

The role of insects in supporting fisheries populations in streams is common knowledge nowadays. It takes many insects to feed many fish. Biologists have known for many years that the biomass of insects in any given stream, at any given moment, is theoretically insufficient to support the number and biomass of co-existing fishes. This has been called the Allen Paradox. This theoretical conundrum was eventually resolved by accounting for the fact that insect populations replace themselves very frequently because many species have short generation-times and high reproductive capacity, yielding a total annual insect production that is much greater than the amount insect biomass observed at any point in time. Thus, it is not just the ‘hatches’

avored by anglers, but the collective diversity, abundance and production of all aquatic insects that provide the food enabling streams to support dense populations of large fish.

## AQUATIC ENTOMOLOGY AND ENVIRONMENTAL MONITORING

The importance of insects in streams extends beyond their roles as fish food and includes critical roles in ecosystem function. Hence, aquatic insects are often used as indicators of ecosystem function and health (e.g., Patrick 1949). Over the years aquatic invertebrates have been used to quantify changes in aquatic ecosystems in many ways, using methods that are specifically developed to answer specific questions. Invertebrates do not comprise the only assemblage of animals used as environmental monitors, but for most investigators they are the best single assemblage. Of the three most commonly used assemblages used to monitor water quality (fish, insects, diatoms), they offer optimal response scales. Insects offer an ideal integration period for water quality at the most relevant timescale for resource management—annual integration. Fish live longer and integrate conditions over many years; it can be difficult to assess the effects of specific years using exclusively taxonomic abundance data. Diatoms complete a life-cycle so fast that they must be sampled many times per year to attain an average annual integration of water quality conditions. But aquatic insect lifecycles are *often* completed in a single year (or two years), allowing their species composition to be used to integrate the conditions over an annum if sampled correctly.

Aquatic insects are also ideal because their limited mobility allows their taxonomic composition to be used to locate disturbances, or to delineate the extent of ecological perturbation. Fish are much more mobile and recolonize a habitat several times between intermittent disturbances. This makes their taxonomic composition useful for watershed scale assessments, but also limits their use in locating smaller stream sections subject to intermittent disturbances.

Insects also comprise the most diverse animal assemblage commonly used for ecological monitoring of water quality and habitat quality. When using taxonomic abundance data, diversity is analogous to resolution in a photograph. Each species has a unique set of environmental tolerances and preferences. There are more species of aquatic insects in most American rivers than there are fish or diatom species.

## METHODS

### FIELD

The Wood River Land trust collected five replicate macroinvertebrate samples from each of six sites in the spring of each year (Table 1). Many macroinvertebrate monitoring programs focus on late summer or early fall because the weather is more conducive to collection of samples from many sites. However, winter or springtime offers several advantages over this period. Most importantly, communities can be sampled before the emergence of most aquatic insect species. This reduces a natural source of variation; the timing of most insect emergences is temperature related. Thus, even if you sample in August or September on precisely the same date each year, some species might be absent or present due exclusively to the accumulation of slight differences of temperature. Thus, spring sampling reduces this source of error while collecting all species that live and survive in the river. Even so, since this is most-complete species list for each stream, all environmental effects will be represented in the community structure data.

The replicate samples were collected using a Hess sampler which encloses a 0.1m<sup>2</sup> sample area and uses a mesh size of 500µm. All rocks were vigorously scrubbed by WRLT personnel and the remaining sand was agitated with all the fine detritus, invertebrates, and moss/algae being collected by the attached capture net. The entire samples were preserved with 99% alcohol and both internal and external labels were attached denoting the date, replicate, and location. The samples were then delivered to the River Continuum Concepts macroinvertebrate laboratory (Manhattan, MT and Butte, MT) for processing and analysis.

In the laboratory, each sample was quantifiably sorted using random aliquots until an excess of 200 random individual invertebrates were attained. These were identified to the lowest possible taxonomic level (usually genus or species), except midges and worms which were identified to family level. Additionally, the samples were subjected to a large-rare search which, after the processing of the quantitative aliquots, required the sorting technicians to spread the entire sample and remove large and unusual specimens for taxonomic analysis. These were combined with the quantitative subsample for some metrics. For the metrics included in this report, only Total taxa richness and EPT Taxa Richness used the Large-Rare-augmented dataset. All percent abundance metrics and the Hilsenhoff Biotic Index used only the aliquots of >200 randomly selected organisms.

**Table 1. WRLT Sampling Sites.** The lotic systems (streams and rivers) at these locations had their macroinvertebrate assemblages quantitatively sampled in the springtime of each year.

SITE	Site Code	LAT	LONG	DRAINAGE_AREA (km <sup>2</sup> )	ELEVATION (m)
Below Magic	BLWMAG	43.2236	-114.354	3901	1424
Stanton Crossing	BWSTANT	43.3292	-114.319	1937	1472
Hailey	BWHAILEY	43.5172	-114.322	1590	1614
East Fork	EFORK	43.603	-114.33	223	1701
Warm Springs	WARMSP	43.6897	-114.385	166	1777
Big Wood River SNRA	BWSNRA	43.7863	-114.425	355	1902

Although the data delivered to the WRLT each year consists of all taxonomic abundance matrices, and an extensive list of ecological metrics, this report focuses only on the metrics detailed below (Table 2). The response of the metrics is also summarized (Table 2) with downward directed arrows indicating that the metric is expected to decrease as the level of anthropogenic disturbance increases. For example, as disturbance increases we usually expect the number of species that live there to decrease. Therefore, the metric Taxa Richness is expected to decrease as water quality or habitat quality decline. Conversely, we anticipate the median-weighted tolerance of insects (i.e., the HBI) and relative abundance of non-insects to increase as water quality and habitat quality decline.

In this brief summary report, no formal statistical testing was performed. However, the graphical analysis includes 95% confidence intervals which will allow readers to gauge the metric means relative to the amount of variance expressed by each metric at each location (or date).

## METRICS, STATISTICS, AND TERMINOLOGY

This report was designed to specifically examine a few metrics that summarize changes in the overall community<sup>1</sup> structure of benthic fauna in terms of their response to disturbance and water quality. The metrics were selected *a priori* because they can often help explain and quantify changes in the overall “health” of streams and rivers. A table describing the expected response of metrics is provided (Table 2), but the discussion is expanded here to promote understanding to the public that may not be familiar with some terminology.

Two of the metrics are taxa richness measures. Taxa richness is the number of different “species” occurring in a sample, but these “species” may not be true biological species—for example they may constitute a family, or genus. Taxa is a plural term describing a number of taxonomic units, the singular of which is a taxon. Thus, the family Chironomidae is a taxon but so is the species *Ephemerella excrucians*—both of which were taxa commonly encountered in the Wood River Basin monitoring study. Taxa richness is a count of the number of taxa.

The rationale for the use of taxa richness measures is simple; fewer species can usually tolerate disturbed or polluted ecosystems. The two-richness metrics used were Total taxa richness and EPT Taxa Richness. Total taxa richness is the sum of all species found in each sample, including the Large/Rare search. Throughout this report the term EPT is used to refer to the insect orders Ephemeroptera, Plecoptera, and Trichoptera. These are the mayflies, stoneflies and caddisflies (respectively) and they are the hallmarks of trout streams. They also tend to be more pollution-intolerant than many other groups of macroinvertebrates. When the text or a figure refers to EPT (as a group, as a richness measure or in terms of abundance) we are referring to a subset of benthic community that is comprised of these aquatic insect orders and is generally considered more sensitive than some others.

Many of the other metrics are relative abundance measures. Relative abundance of a group is an estimate of its proportional abundance in the community. For example, if we found 1000 macroinvertebrates in a sample and 500 were midges, the midge relative abundance would be 50% of community. This is distinct from density which would be expressed as the number of animals per unit area (e.g., per sample or per square meter). Density is discussed only occasionally as it was beyond the scope of this report.

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<sup>1</sup> Biological communities are groups (assemblages) of interacting populations of different species.

The last metric considered in this report is the Hilsenhoff Biotic Index (HBI). It is a weighted average of the pollution tolerance of the invertebrates sampled. The metric varies between zero and 10, with high HBI values indicative of dominance by tolerant species; whereas low value is cause by dominance of invertebrates that require cleaner water. The metric was developed to gauge the impacts of organic pollution in Wisconsin rivers, but it is responsive to a broader range of impacts that often accompany organic pollution (e.g., sedimentation, temperature elevations, salinity and others). Additionally, values have been amended to allow broader regional application of the method (e.g., Barbour 1999). Nonetheless, note that it was gauged to develop a way to quantify the impact of untreated or poorly treated sewage on stream communities. Therefore, his classifications of “fair” or “good” conditions might not be the scale we expect for a western trout stream. The metric is usually an excellent integrator of change in terms of the role of sensitive species and their displacement. That is, even if the classification scheme of good, fair and poor are not locally accurate, the metric is an excellent way to describe changes (in time, or geographically) in the health of streams. This metric is used in several ways in this report, and they are discussed as used.

The statistical methods used for this report were very limited and simplified. Averages of metrics and the associated 95% confidence intervals were used to compare sites. Thus, the error-bars presented around the average metric values in each section represent the expected range of the true average value with 95% confidence. There was a wide range of variation in confidence intervals and relaxing the 95% criterion ( $\alpha=0.05$ ) to 90% ( $\alpha=.0.10$ ) would increase our ability to describe changes. This visual comparison style does not account for deviations from normality, significant interactions, or variance heterogeneity; and was meant to be a screening of the data for trends. Performing formal statistical testing procedures is possible, but beyond the scope of this report. Additionally, given the variances observed these procedures may not have elucidated changes any better than the methods we used. In the event that these results are to be published in the future, the analyses can be expanded.

**Table 2. Metrics Used in This Report.** This is a subset of metrics that are provided to WRLT each year. Metrics with the denotation “LR” had the taxa lists augmented with the large/rare search procedure. Metrics denoted with “Q” only used quantitative random subsamples.

<b>Metric Class</b>	<b>Metric</b>	<b>Hypothesized Response to Anthropogenic Disturbance</b>	<b>NOTES</b>
Ecological Community Metrics	Taxa Richness (LR)	↓	Total number of “species”
Community Stress Metrics	EPT Richness (LR)	↓	The number of “species” from the orders Ephemeroptera, Plecoptera, and Trichoptera
	EPT Relative Abundance (Q)	↓	The proportion of animals in the sample that belong to the EPT orders (e.g., above)
	Chironomid Midge Relative Abundance (Q)	↑	The proportion of animals in the sample that are chironomid midges
	Non-Insect Relative Abundance (Q)	↑	The proportion of the animals in the sample that are not insects.
	Hilsenhoff Biotic Index (Q) (HBI)	↑	The abundance-weighted average organic* pollution tolerance of all organisms in the sample.

## RESULTS: CHANGES OVER TIME AT EACH LOCATION

### CHANGES BELOW MAGIC (BLWMAG)

Both Total taxa richness and EPT richness did not appear to change significantly among years as there was significant overlap in the confidence intervals of the metric each year. Both metrics expressed greater mean richness, in 2022, with an apparent decline in successive years. However, this apparent “trend” was strongly influenced by an outlier sample with greater richness than the other samples.

The relative abundance of mayflies, stoneflies, and caddisflies (EPT) did not appear significantly different among years due to significant overlap in abundances among years. Although the average relative abundance of EPT species was relatively low, some samples were comprised of >50% EPT in 2022 and 2024.

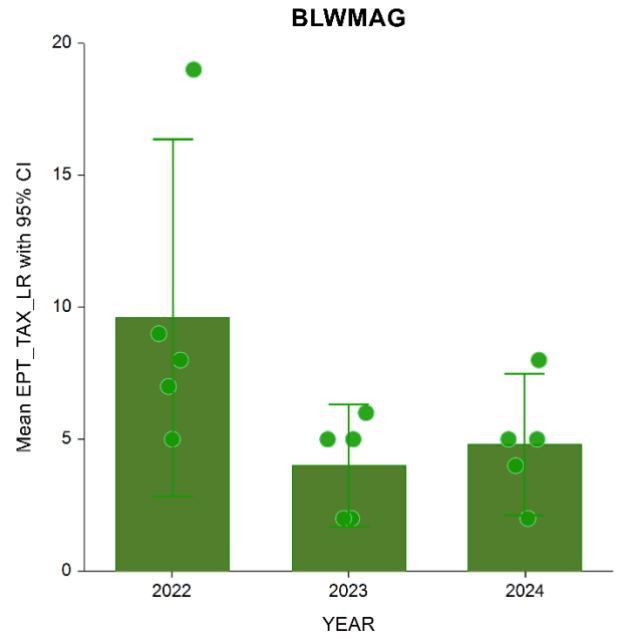
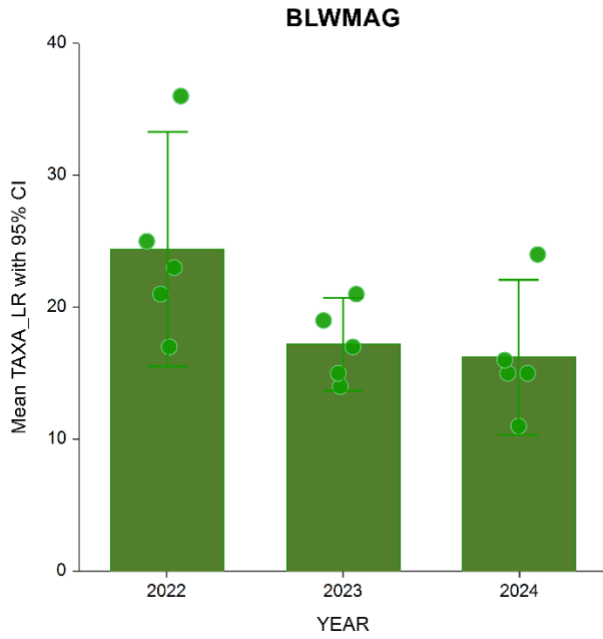
The relative abundance of midges and non-insects offset changes in EPT abundance. That is, the relative abundance of EPTs were low in 2022 because midge abundance was elevated, whereas the EPT abundance was lower in 2023 because the relative abundance of non-insects was elevated. Both the relative abundance of midges and non-insects had a wide range of variation. The specific midges dominating samples in 2022 (comprising up to 80% of the sample) remain unknown because midges were not identified below the family-level for this project, but high densities of filter feeders (e.g., *Rheotanytarsus* sp) may have dominated if there was a source of suspended fine particles upstream (such as an impoundment, treatment facility, or riparian livestock). Otherwise, both midges and non-insects (worms) could increase abundance if habitat provided refuge—both groups can become abundant among sediment or among the branches of mosses and filamentous algae.

The benthic assemblages appeared to support significantly fewer non-insects in 2022 than in 2023 but did not appear significantly different from 2024’s non-insect abundance. Except in unusual circumstances, we usually expect non-insects to comprise less than ~20% of the benthic assemblage. The only year with an average non-insect abundance below 20% was 2022, but 2024 had one sample with abundances of non-insects below ~20%.

The HBI values were strongly affected by midge abundance in 2022. Midges as a family received a collective tolerance value of 6<sup>2</sup>, and the samples from 2022 were clustered around the mean HBI of 5.94. In 2023, the HBI ( $\bar{x}$  = 6.82) was pulled upward by oligochaete worms, predominantly *Nais* sp., which has a tolerance value of 9.1. In 2024, HBI was lower (4.78)—the only year with mean HBI < 5.0—because both midges and non-insects exhibited lower abundance which Hilsenhoff classified as “good.”

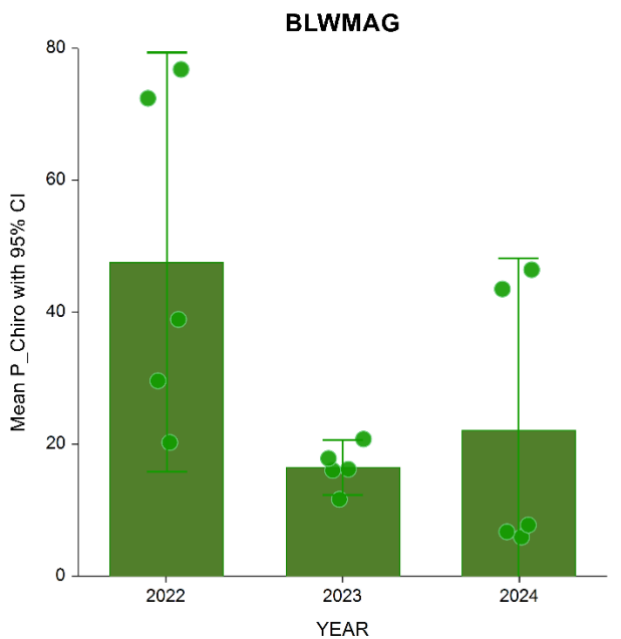
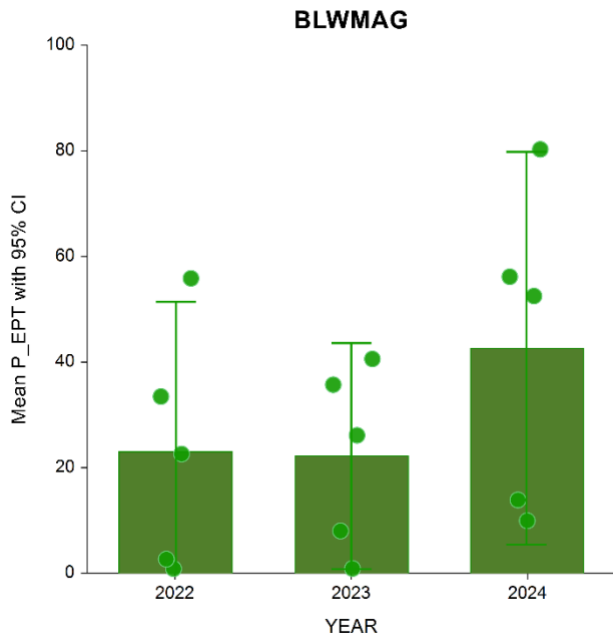
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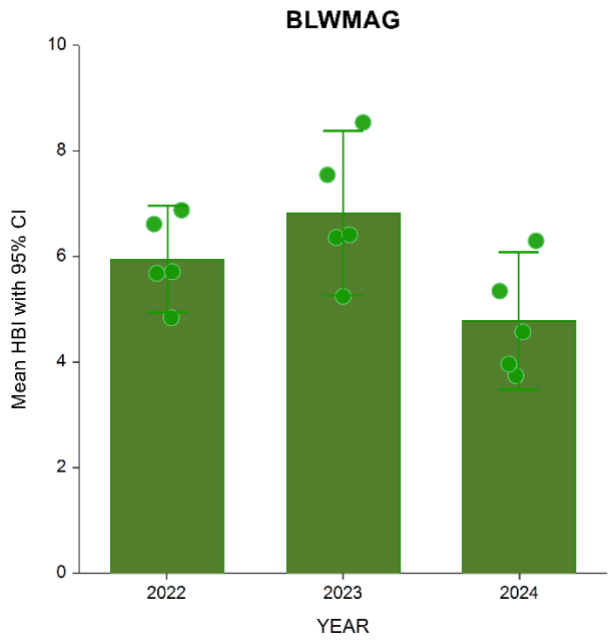
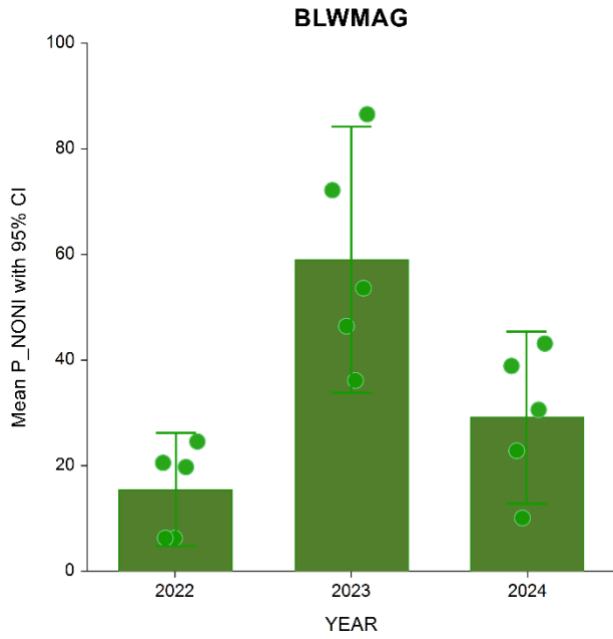
<sup>2</sup> However, the genera of the Chironomidae have been assigned a wide range of tolerance values—from very sensitive, to facultative, and very tolerant. Thus, a heavily abundance-weighted HBI score of 6 may be related to taxonomic effort.



Total taxa richness, and EPT richness ↑

Relative Abundance (%) of EPT and of Midge larvae ↓









## CHANGES AT STANTON CROSSING (BWSTANTON)

Both Total taxa richness and EPT richness exhibited the same pattern among years. That is, the highest mean value was observed in 2022, the lowest mean in 2023, and the median in 2024. However, the high mean value was only significantly greater than the 2023 observation for Total taxa richness. In the Greater Yellowstone Area / Intermountain West, EPT richness values of < 9.0 are disconcerting using these laboratory and field methods. The average EPT richness value at BWSTANTON was 9.0 in 2023 (with two samples containing fewer than 9 EPT taxa). For perspective, most urban streams have EPT richness values between 0-4 EPT taxa, thus there is room for improvement, but the monitoring program should still be able to detect declines.

Mean EPT relative abundance appeared greatest in 2024. However, a wide range of overlaps among confidence intervals suggests that these differences were not statistically significant—2024 samples ranged from 19.6% to 88.0% of the community. Nonetheless, 2024 appeared to represent a marked recovery in the abundance of EPT taxa; with three samples comprised of >60% EPT's. The wide range of variation suggests that a wide range of habitat quality were sampled—either as substrata diversity, flow diversity, or both. The improvement in 2024 may have been related to improving flow conditions—perhaps from the previous year.

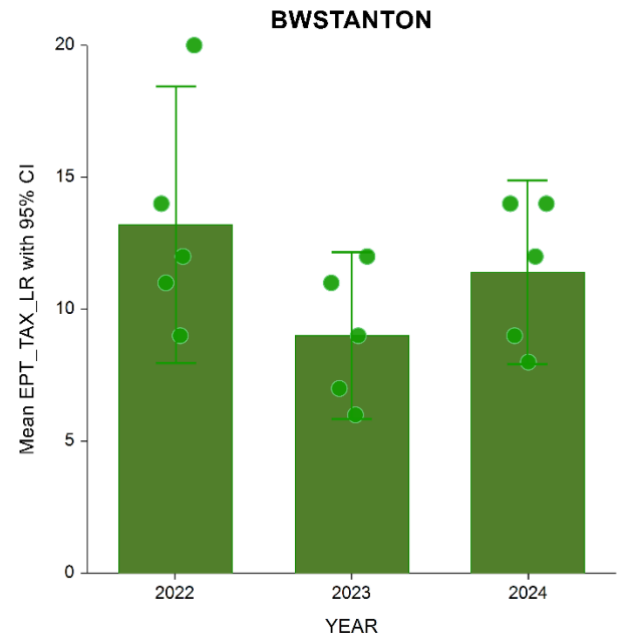
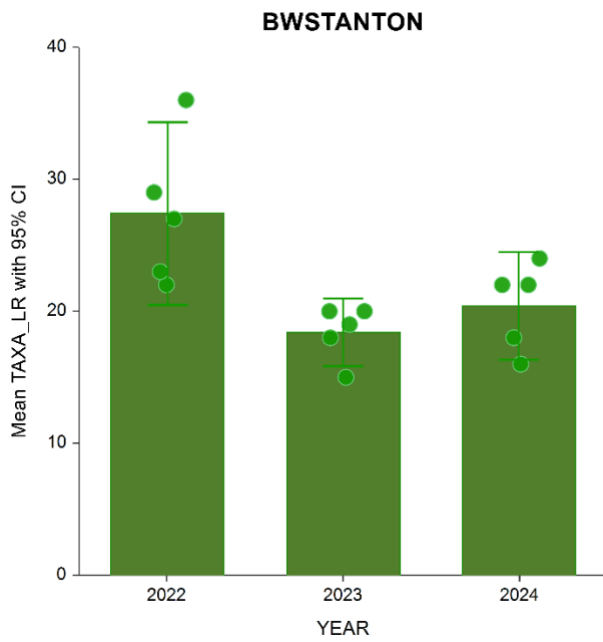
EPT taxa richness exhibited much more overlap among confidence intervals (Figs.), but did not appear to change significantly among years as there was significant overlap in the confidence intervals of the metric each year. Both metrics expressed greater mean richness, in 2022, with an apparent decline in successive years. However, this apparent “trend” was strongly influenced by an outlier sample with greater richness than the other samples.

The relative abundance of chironomid midges steadily increased each year but was generally below one third of the community—except for two samples collected in 2024. This is typical for the region.

In the spring of 2022 and 2023, a disproportionately high abundance of non-insects dominated the site. These two years were not significantly different from each other, but during both years, the benthic communities exhibited significantly greater non-insect abundance than observed in 2024. This is usually due to sediment accumulation, which may have been retained among aquatic plants (algae/moss) or by reduced flow. If depositional areas were sampled, then it may also reflect localized erosion. The consistently low non-insect abundance ( $\bar{x}$  = 5.1%, range 0.44-14.1%) in 2024 represents a marked improvement to the site.

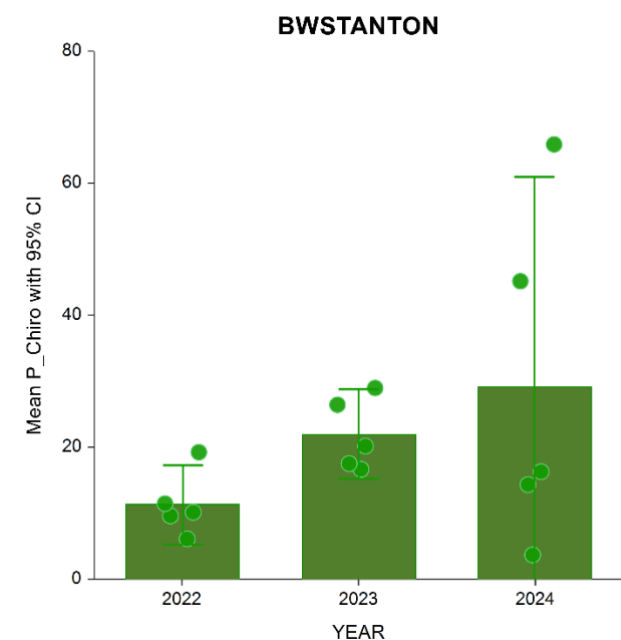
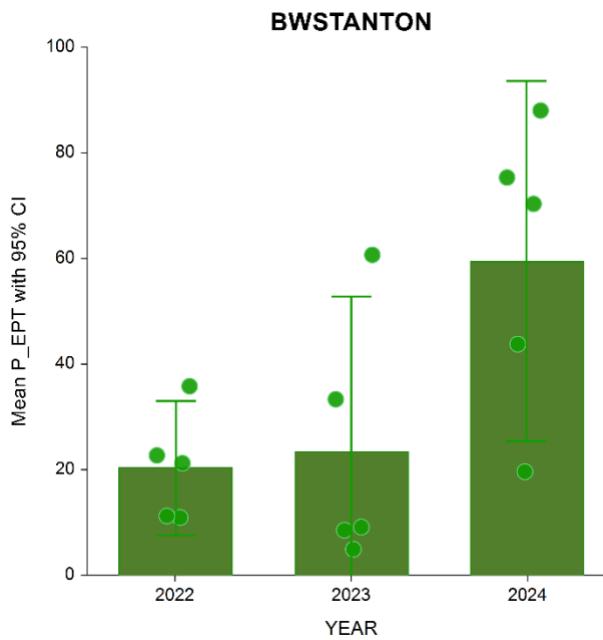
The high HBI values of 2022 ( $\bar{x}$  = 7.11, “fairly poor”) and 2023 ( $\bar{x}$  = 7.02, “fairly poor”), were most strongly related to high abundances of worms pulling the values upwards. The reduction of worm abundances in 2024 improved HBI values markedly ( $\bar{x}$  = 3.21 “excellent”).

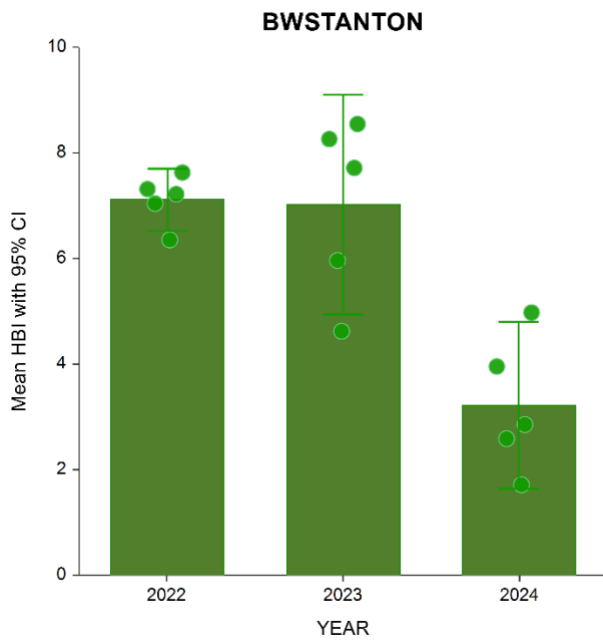
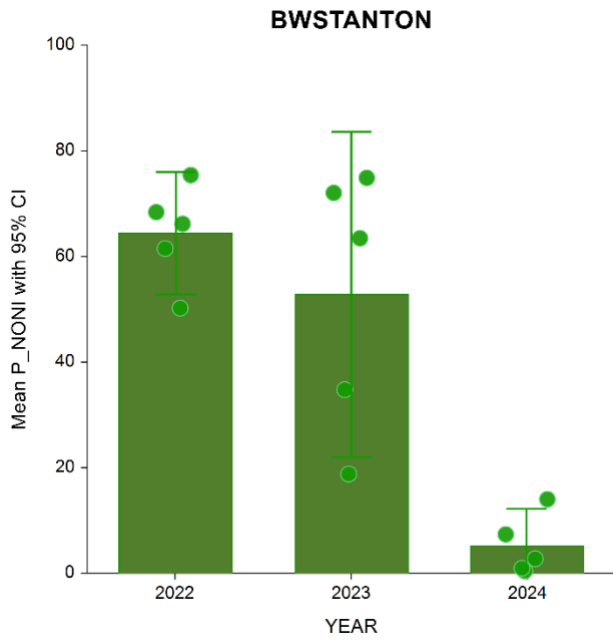
Although HBI scores improved, this site still exhibited relatively low richness of EPT taxa—even in 2024. Nonetheless, even with an increase in average midge abundance in 2024, the abundance of non-insects in that year constitutes an improvement at the end of the current monitoring period.



Total taxa richness, and EPT richness ↑

Relative Abundance (%) of EPT and of Midge larvae ↓







## CHANGES AT BW: HAILEY (BWAHAILEY)

Total taxa richness appeared significantly lower in 2023 than in 2022, but 2024 did not appear significantly different from either 2022 or 2023.

EPT richness did not appear to change significantly between 2022-2024. Samples collected from all years (2022-2024) had mean EPT richness values >9.0—if only barely; 2023 had an average EPT richness of 9.6. In 2024, four of the five samples collected reflected an EPT richness >10.0, which could represent a modest improvement. Nonetheless, with significant overlap among the confidence intervals, any changes—improvement or otherwise—were small.

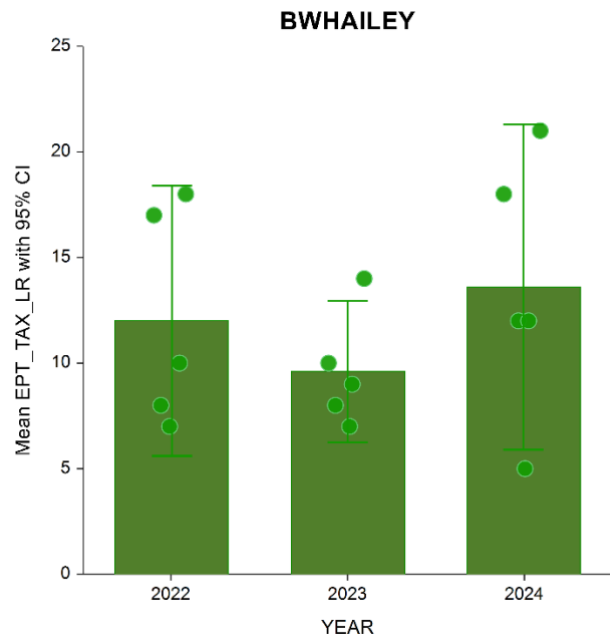
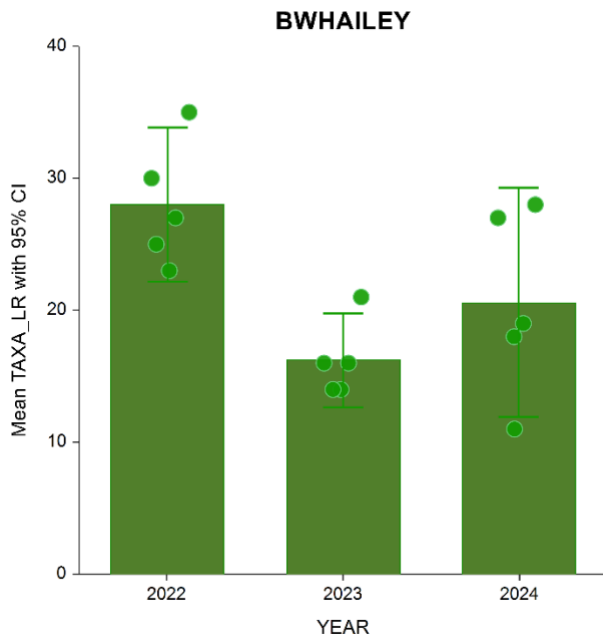
The relative abundance of EPT in 2024 was significantly different from 2023, but none of the other years were significantly different from each other. The wide confidence intervals in 2022 were due to one sample having abnormally low EPT relative abundance—otherwise the values were very similar to those attained during the 2023 sampling period.

The relative abundance of chironomid midges was in the “normal” range for the region in 2022 and 2023 but was a little higher than expected in 2024—which had significantly higher average chironomid abundance than observed in 2023, but was not significantly different from 2022 due to wide confidence intervals.

Most samples collected had a low relative abundance of non-insects, with most samples generating values in the ideal range of less than 20%. However, in 2022 slightly higher values and one outlier with 87.6% raised the average non-insect relative abundance greater than 20% (27.5%).

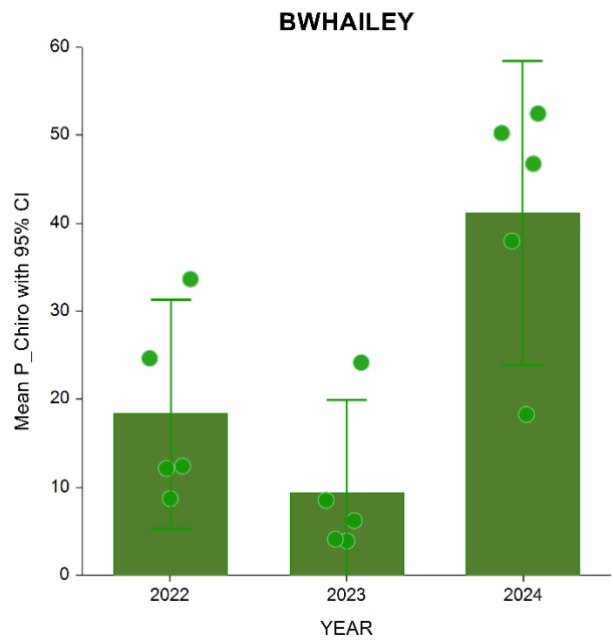
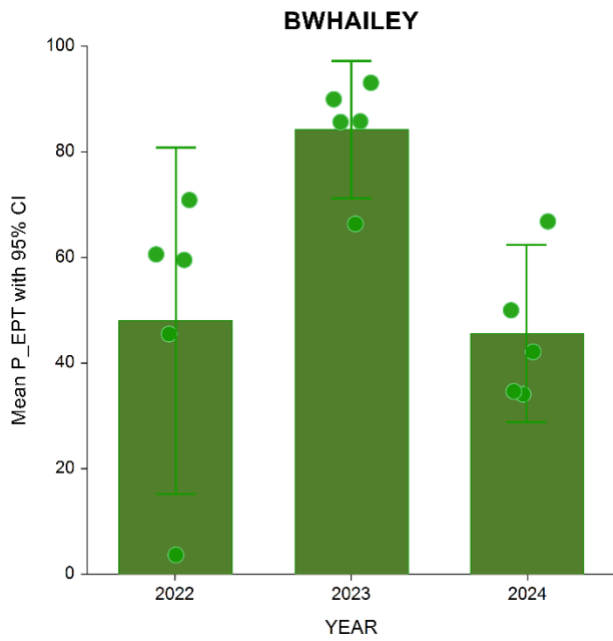
The HBI values attained from samples collected in 2022 included one outlier with HBI values >6 (“Fair”), but nonetheless, mean HBI values remained less than 4.0 in all years and would be classified by Hilsenhoff (1987) as “very good,” on average. However, in 2023, the community at Hailey yielded an average HBI of 1.76 (“excellent”), which underscores the numerical dominance of EPT taxa in 2023—which had elevated mayfly densities compared to other years (including higher abundances of *Rhithrogena*, *Epeorus*, and two species of *Drunella*).

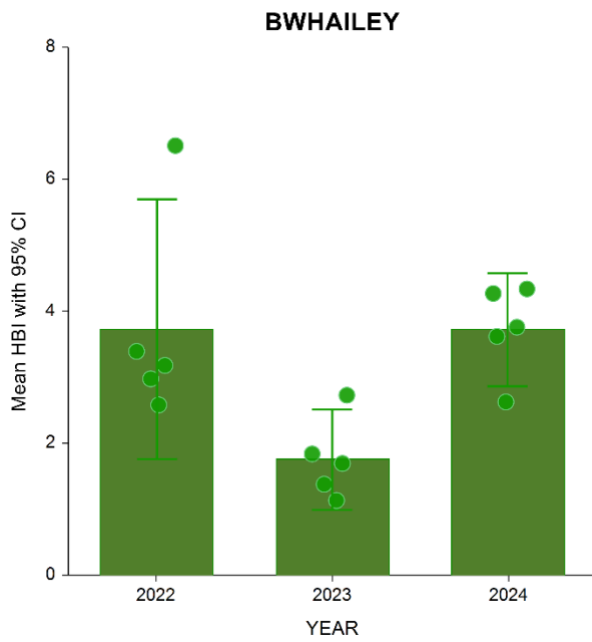
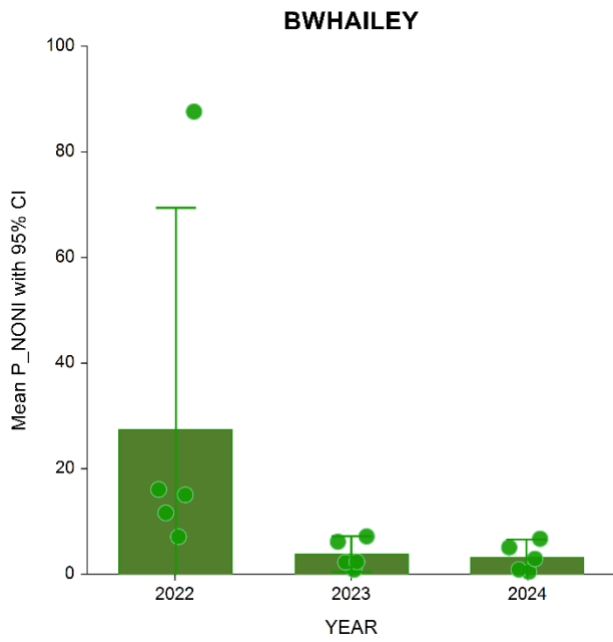
Although one sample from 2022 had elevated worm densities, this did not translate into reduced richness of the assemblage in terms of Total taxa richness and EPT Taxa Richness. Worm abundance affected the range of confidence intervals of all the relative abundance metrics in 2022. Elevated average midge abundance in 2024 did not significantly reduce Total Richness or EPT richness. It may have resulted in slightly higher HBI values in 2024 compared to 2023, but all years had average HBI values less than 4.0. Generally, the condition of benthic assemblage at BWAHAILEY has held steady over the three years of this assessment.



Total taxa richness, and EPT richness ↑

Relative Abundance (%) of EPT and of Midge larvae ↓









## CHANGES AT EAST FORK (EFORK)

East Fork showed no significant differences in Total taxa richness over the sampling period. However, average EPT richness appeared to increase in 2024 (the differences among years do not appear statistically significant due to significant overlap of confidence intervals). Average EPT richness was lower than desired in 2023 but did not constitute a significant deviation from prior years.

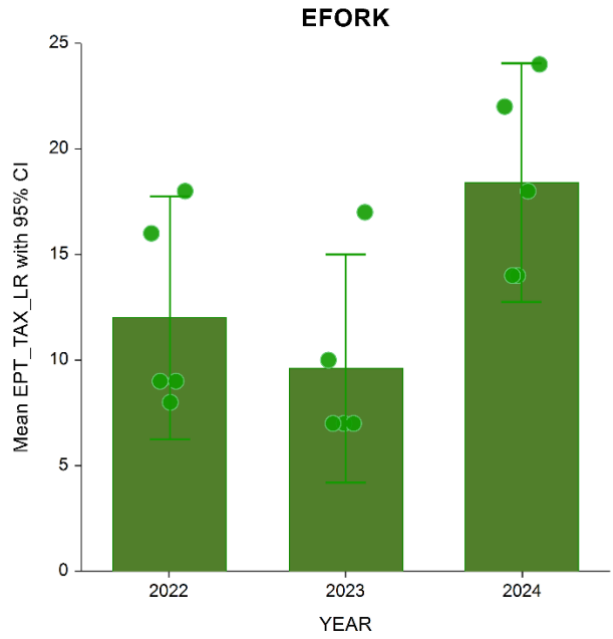
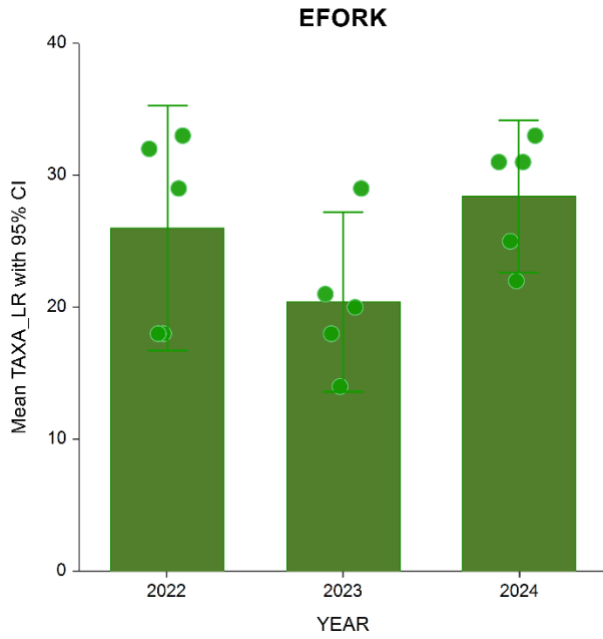
The relative abundance of EPT taxa appeared to gradually increase, incrementally, over time—which could constitute a gradual improvement. However, due to the wide confidence intervals and the scale of any changes, the differences among specific years were not statistically significant. Nonetheless, the relative abundance of EPT taxa indicated showed no significant decline

Chironomid midges should generally comprise about 10-35% of the benthic assemblage in smaller rivers in the intermountain regions. There are numerous natural reasons why this might not happen. Nonetheless, except for one outlier in 2022, the relative abundances of midges remained in the range of these expectations.

The average relative abundances of non-insects remained relatively constant over the term of the monitoring program. The average values remained below the threshold of concern for the entire monitoring program, and therefore did not indicate any detrimental shifts in the community during the term of the monitoring program.

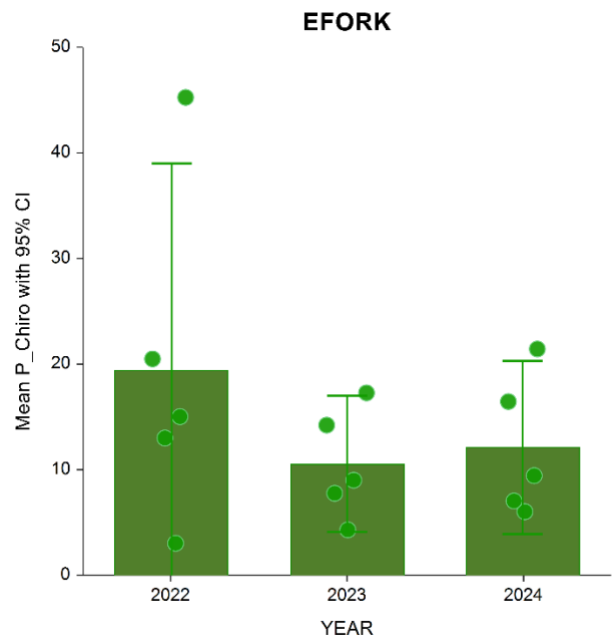
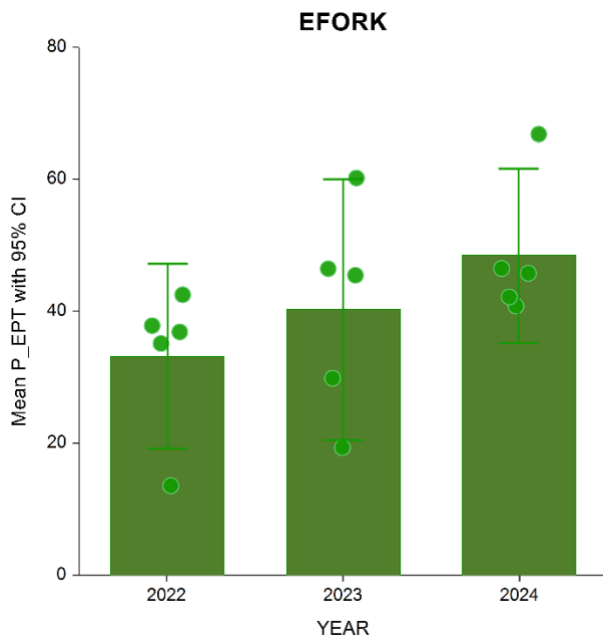
The average Hilsenhoff Biotic (HBI) Index values gradually declined over the term of the monitoring program. Although there were no statistically significant differences among the years sampled, the averages moved from Hilsenhoff's (1987) "good" water quality classification into the "very good" category. This reflects the net effects of the increase in EPT richness and EPT abundance, in addition to a slight decline in the abundance of midges and worms in 2024.

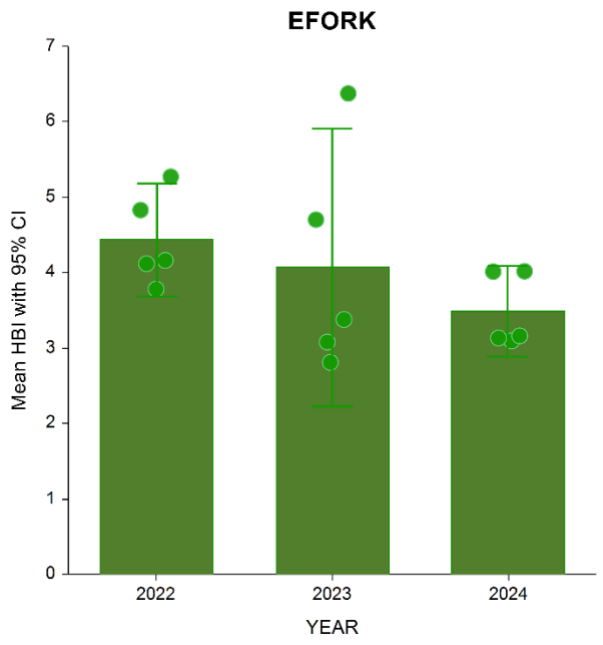
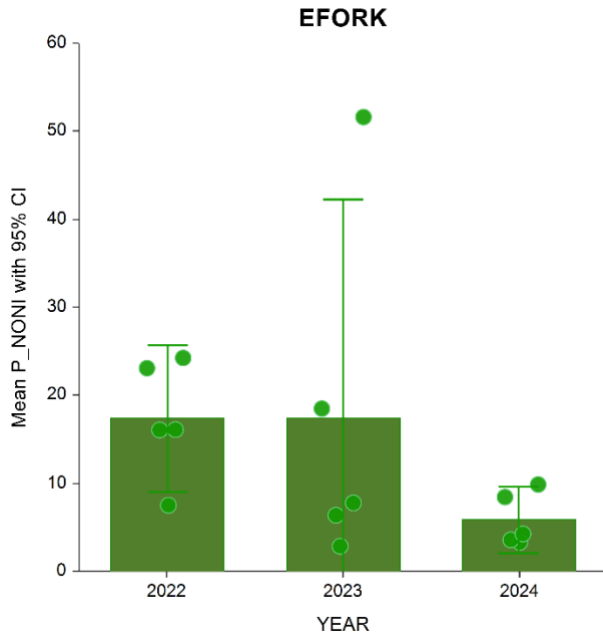
The site did not show any significant declines in quality over the monitoring period and may have exhibited minor improvements in 2024. Although the confidence intervals did not indicate that any of the changes were statistically significant (at  $\alpha=0.05$  level), the response signatures among EPT richness, EPT abundance, Chironomid abundance, non-insect abundance and HBI were all congruent with a relatively stable environment and could reflect modest improvements relative to 2022.



Total taxa richness, and EPT richness ↑

Relative Abundance (%) of EPT and of Midge larvae ↓







## CHANGES AT WARM SPRINGS (WARMSP)

Both Total taxa richness and EPT richness expressed a community with significantly greater values in 2024—as compared to 2022. The richness of EPT's appeared to shift more incrementally than the increase in Total taxa richness—However, the only statistically significant difference in EPT richness occurred between the samples collected in 2022 and 2024, with 2023 not exhibiting distributions that were statistically significantly different from either 2022 or 2024. Average EPT richness was abnormally low in 2022. Total taxa richness in 2024 was significantly greater than observations from 2022 and 2023. Both metrics could constitute a statistically significant improvement over the previous year.

The relative abundance of EPTs increased from very low in 2022 to very high over the term of the monitoring program. Due to high variability the EPT abundances of 2023 were not significantly different from 2022 or 2024. However, EPT's consistently comprised a significantly proportion of the community in 2024 than they did in 2022, and possibly 2023.<sup>3</sup> Although EPT's are generally desirable, an elevated average EPT abundance (~>80%) can indicate certain problems. For example, if a stream is subject to frequent intermittent disturbances or the streambed was recently inundated, then a very high abundance of EPT's might indicate that rapid colonizer taxa (e.g., *Baetis* sp.) were present, whereas slower colonizers were absent. When this happens, we expect to observe very high EPT relative abundance but very low EPT richness. However, the EPT richness was also very high and proportional to EPT relative abundance. Thus, WARMSP exhibited a diversity of EPT species in 2024, suggesting the high EPT abundance was not the result of disturbance and low diversity. A supplementary figure is provided (below), showing the average density of all mayfly species during each year—this clearly shows a dramatic increase in cool stenotherm (as well as clinger taxa) insects in both 2023 and 2024.

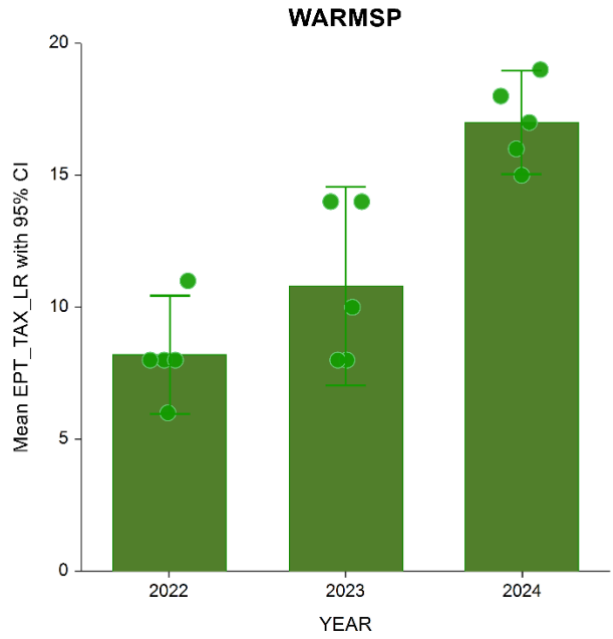
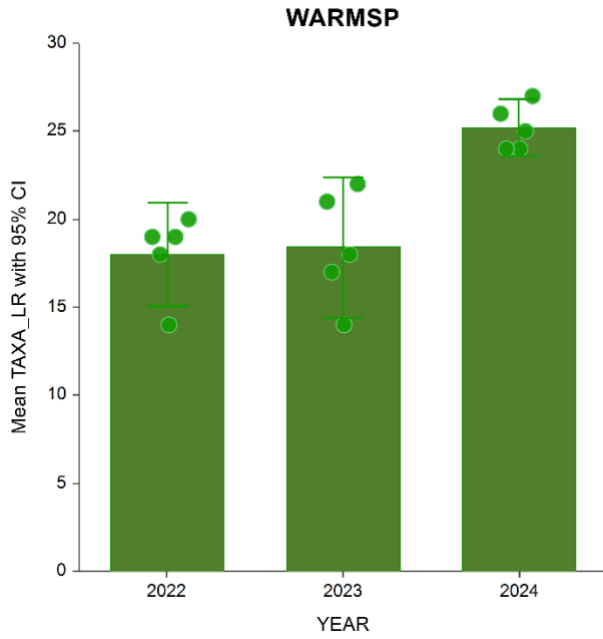
The primary reason for low EPT relative abundance in 2022 was the dominance of chironomid midges—which comprised >50% of the community on average—with two samples describing a community more than 60 dominated by Chironomidae. Samples collected in 2023 exhibited a more “normal” level of chironomid midge-dominance—and samples from 2024 described a community with abnormally low abundance of midges.

Non-insect relative abundance was low in 2022 and 2024—but above the 20% threshold in 2023. The only significant difference observed indicated that 2024 had significantly fewer (%) non-insects than 2022.

The HBI showed a gradual decrease in the median tolerance of insects to inhospitable conditions over the term of the monitoring period. Although the HBI scores were buoyed by increased mayfly abundance in 2023 and 2024, the higher non-insect abundances of 2023 resulted in an intermediate increase (improvement) in HBI. Thus, the apparent trend shows a gradual improvement in the condition of this site. HBI values changed from averages of 5.06 (2022), 4.00 (2023), and 1.88—from “good” to “very good” and “excellent” in 2024. These changes were primarily driven by the increases in the diversity and abundance of mayflies (q.v., supplemental figure), along with the decreases in the relative abundances of worms and midges.

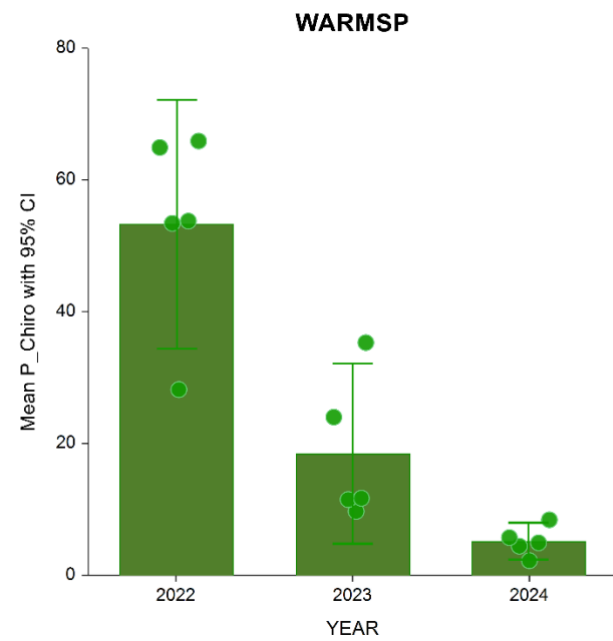
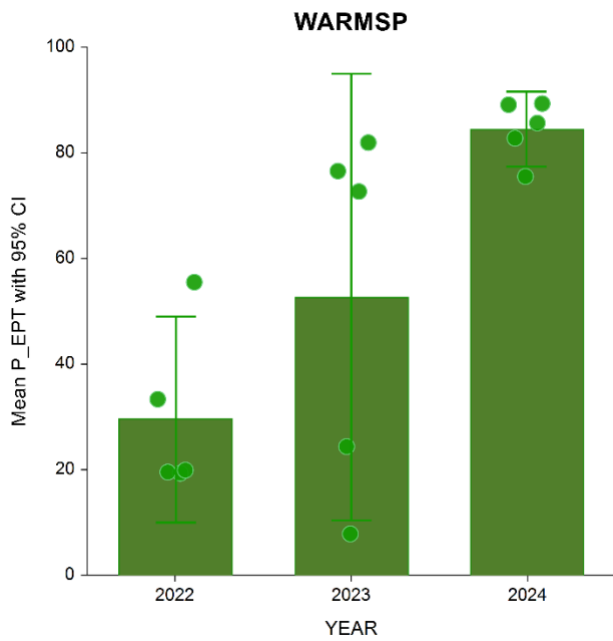
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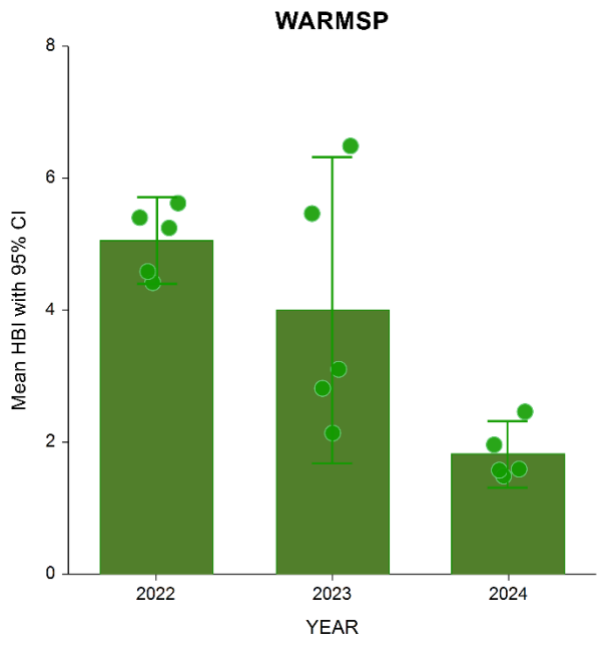
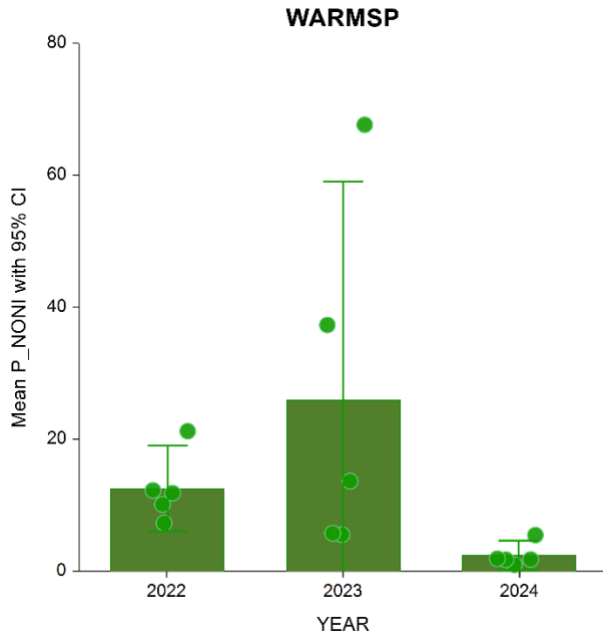
<sup>3</sup> The confidence intervals between the years overlap significantly due to a very wide range of variation in 2023. However, all observations from 2023 were below all observations from 2024. A non-parametric statistical analysis, such as Kruskal-Wallis, would most likely define a high degree of statistical significance even if traditional parametric statistics would not (high variation and coefficient of variation).



Total taxa richness, and EPT richness ↑

Relative Abundance (%) of EPT and of Midge larvae ↓

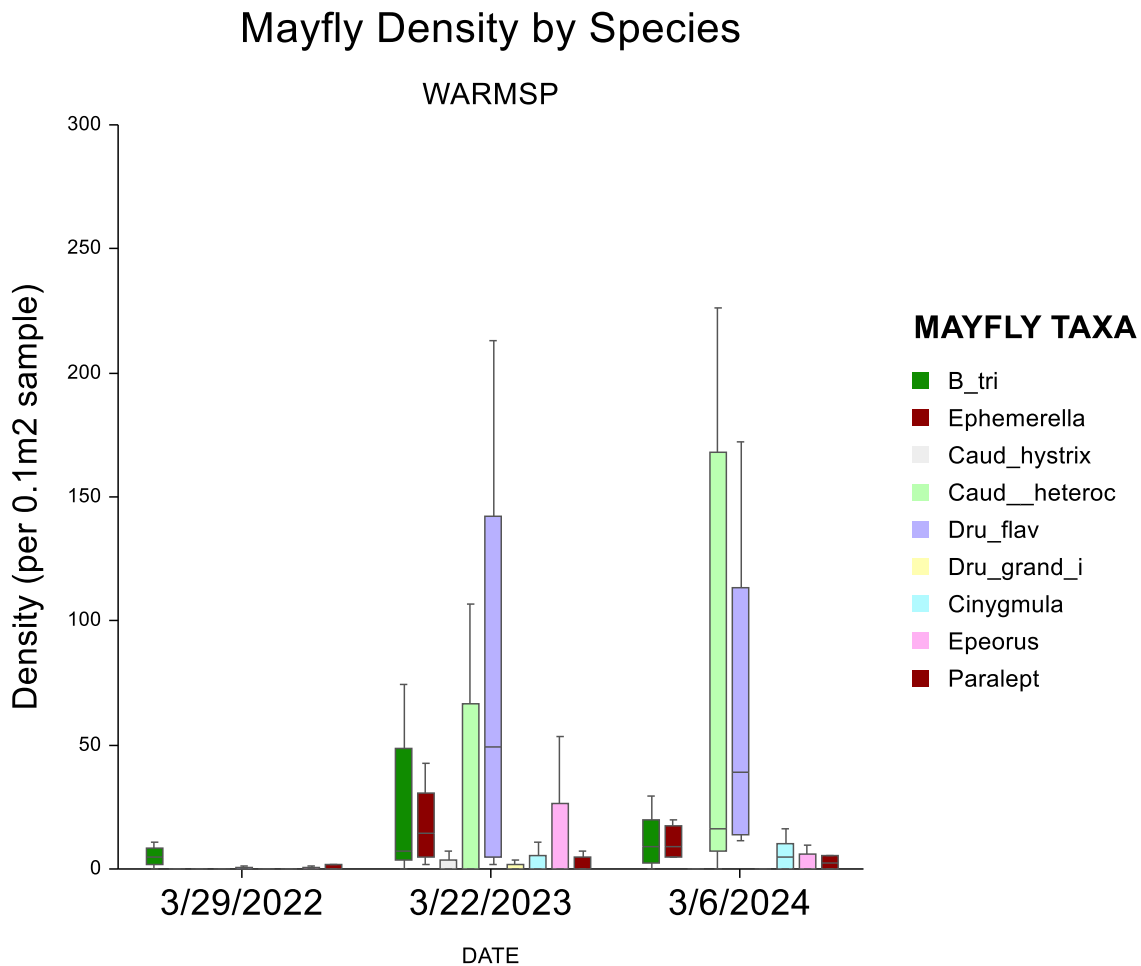






WARMSP SUPPLEMENTARY FIGURE. ↓

This supplementary figure shows how the density (per sample) of all mayfly species changed at WARMSP over time. Notice that in 2022, only *Baetis tricaudatus* was collected from the site consistently (no zero values)—other species had lower abundances and many observations of zero abundance. In subsequent years, the mayfly fauna included variable abundances of *Ephemerella* (PMD), *Caudatella hystrix*, *C. heterocaudata*, *Drunella flavilinea* (Flavs), *D. grandis* (green drake), *Cinygmula*, *Epeorus*, and *Paraleptophleba* (=Neoleptophlebia)



## CHANGES AT BIG WOOD RIVER SNRA (BWSNRA)

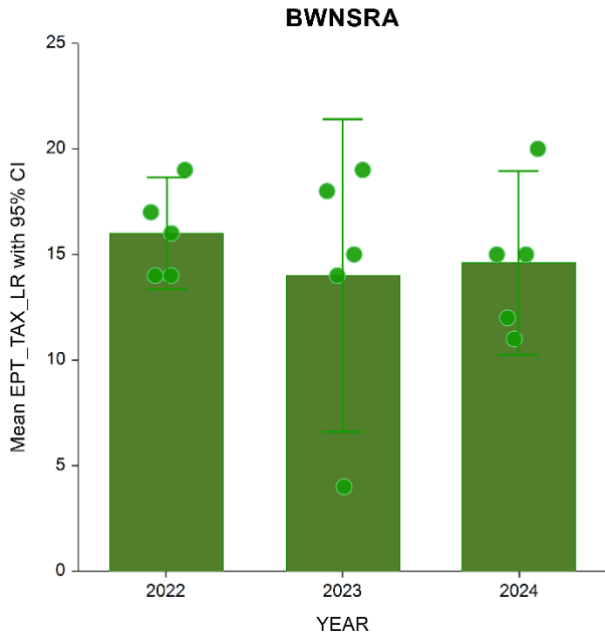
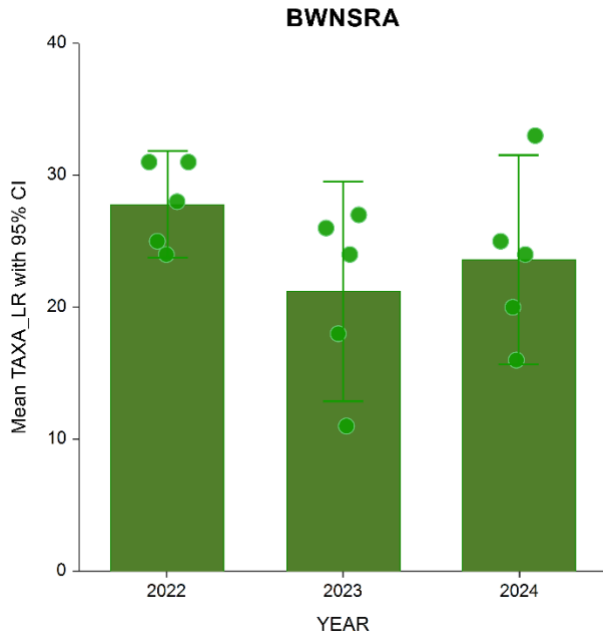
BWSNRA exhibited the greatest consistency in Total taxa richness and in EPT richness. EPT richness remained well above the 9.0 threshold of impairment. One sample in 2023 was an outlier that may have been collected from sub-optimal habitat or from a recently desiccated and re-inundated riffle because it had many fewer EPT species than all other samples during all years. The one outlier notwithstanding, the EPT richness of individual samples and the average EPT richness values were all consistently well above the regional impairment threshold of 9.0.

Average EPT relative abundance remained constantly in the optimal range (~60%) during the monitoring period. However, one sample in 2023 had a much lower EPT relative abundance than all other samples in all years of monitoring. This was the same individual sample that was an outlier for EPT richness in 2023.

The average relative abundance of chironomid midges was in the normal range on average all year. The outlier sample from 2023 was 80% comprised of midges and does not seem representative of the community from which all other samples were collected.

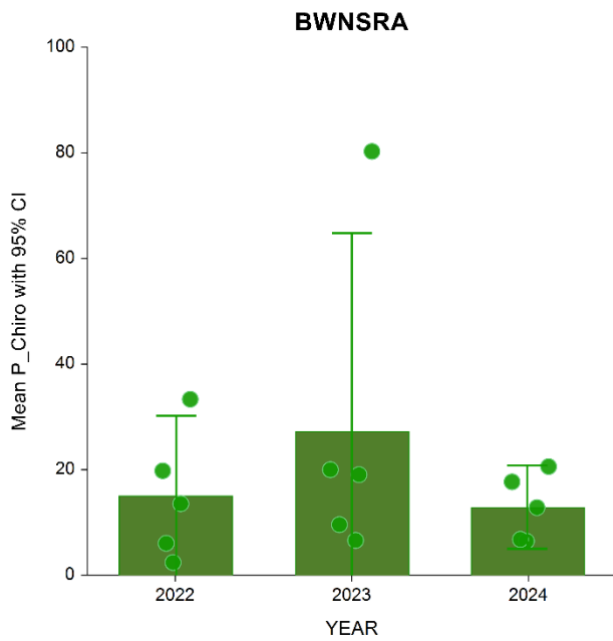
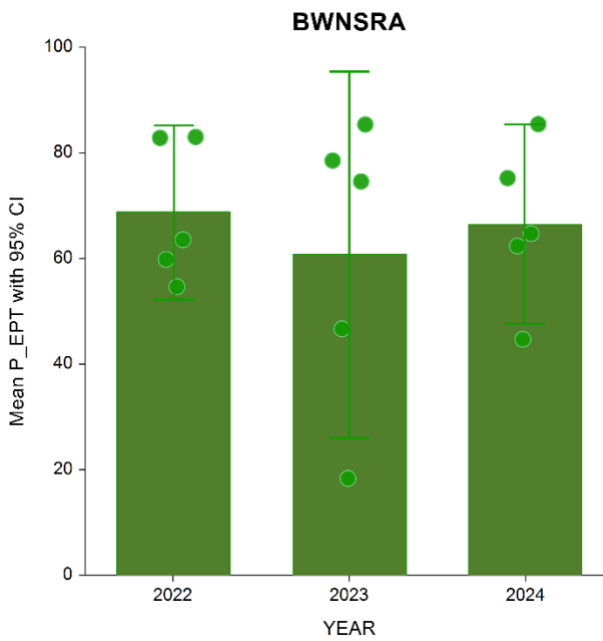
The range of variation in non-insect relative abundance increased dramatically in 2024, with two samples exhibiting much greater abundance of non-insects (>20%). However, most of the samples had very low non-insect abundances, consistent with expectations and with previous years at this site.

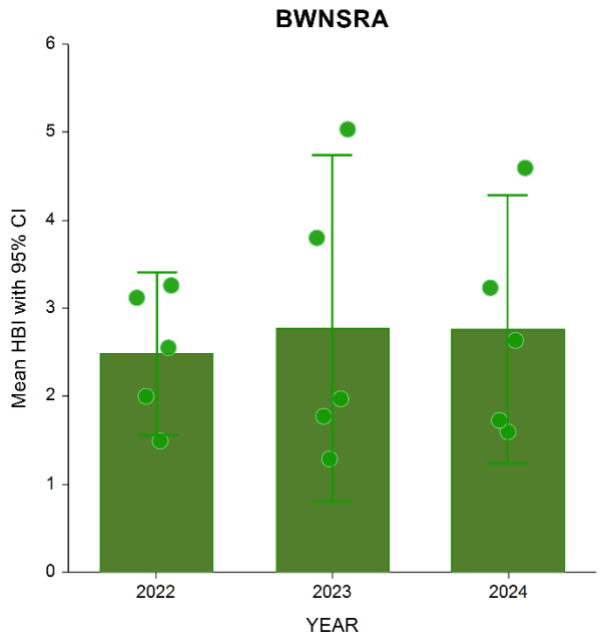
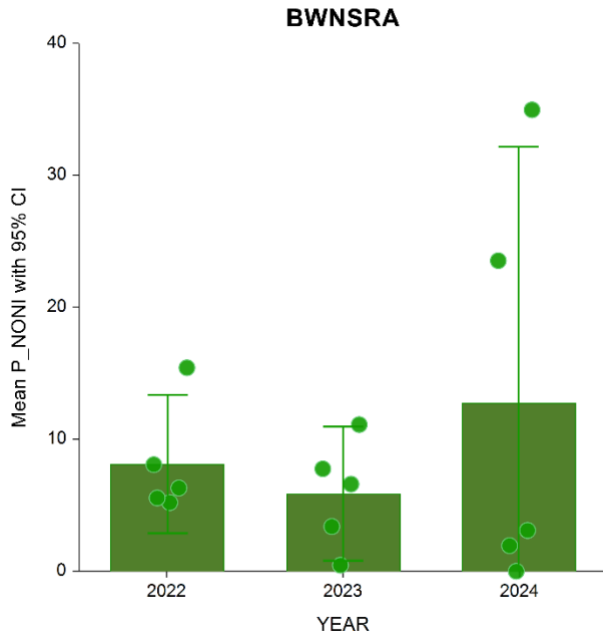
The HBI responded to the net changes of in the benthic communities and indicated that over all, with all the off-setting changes, that there was very little change at BWNSRA over time and that the overall water quality of the site remained very good.



Total taxa richness, and EPT richness ↑

Relative Abundance (%) of EPT and of Midge larvae ↓







## CHANGES AMONG SITES DURING EACH YEAR

The previous section described how each individual site changed over each year. This section describes how all the sites differ from each other each year. This information describes spatial trends, whereas the previous section describes temporal changes. Examination of spatial trends allows resource managers to prioritize sites.<sup>4</sup> For example, if one site consistently has greater non-insect and midge abundances (and HBI) than the others, it might benefit from strategies that reduce organic or sediment loads. Similarly, if one site has exceptional EPT richness compared to the other sites, it might warrant greater conservation efforts. The metrics are presented on different graphs to make interpretation easier; there are three graphs for the metric, one each year, showing the differences at all sites.

## DIFFERENCES AMONG THE SITES TAXA RICHNESS

To someone who uses these methods often, the total taxa richness values may appear somewhat low across the whole basin, but this is because midges were not identified to genus. Midges may add 15-25 taxa to each sample. Considering this, true taxa richness may have been quite high at most sites, especially in 2022 and 2024.

In 2022, total taxa richness was lowest at WARMSP and greatest at BWSTANTON, EFORK, BWSNRA—the higher sites had similar variation and mean magnitude suggesting no real spatial trend. WARMSP was significantly different (lower) than BWSTANT, BWHAILEY, and BWSNRA. The average richness of all observations in 2022 was 25.6 taxa.

In 2023, total taxa richness ( $\bar{x}$  = 18.6 taxa) was lower at all sites (note the difference in scale compared to other years). There were no significant differences among the sites in 2023. Also, recall from the previous section that Total taxa richness at WARMSP remained virtually unchanged in 2023 as compared to 2022. Thus, the similarity among sites in 2023 resulted from a decline in invertebrate diversity across the board (except at WARMSP), not from an increase in diversity at WARMSP. Therefore, any differences among sites were too small to discern statistically.

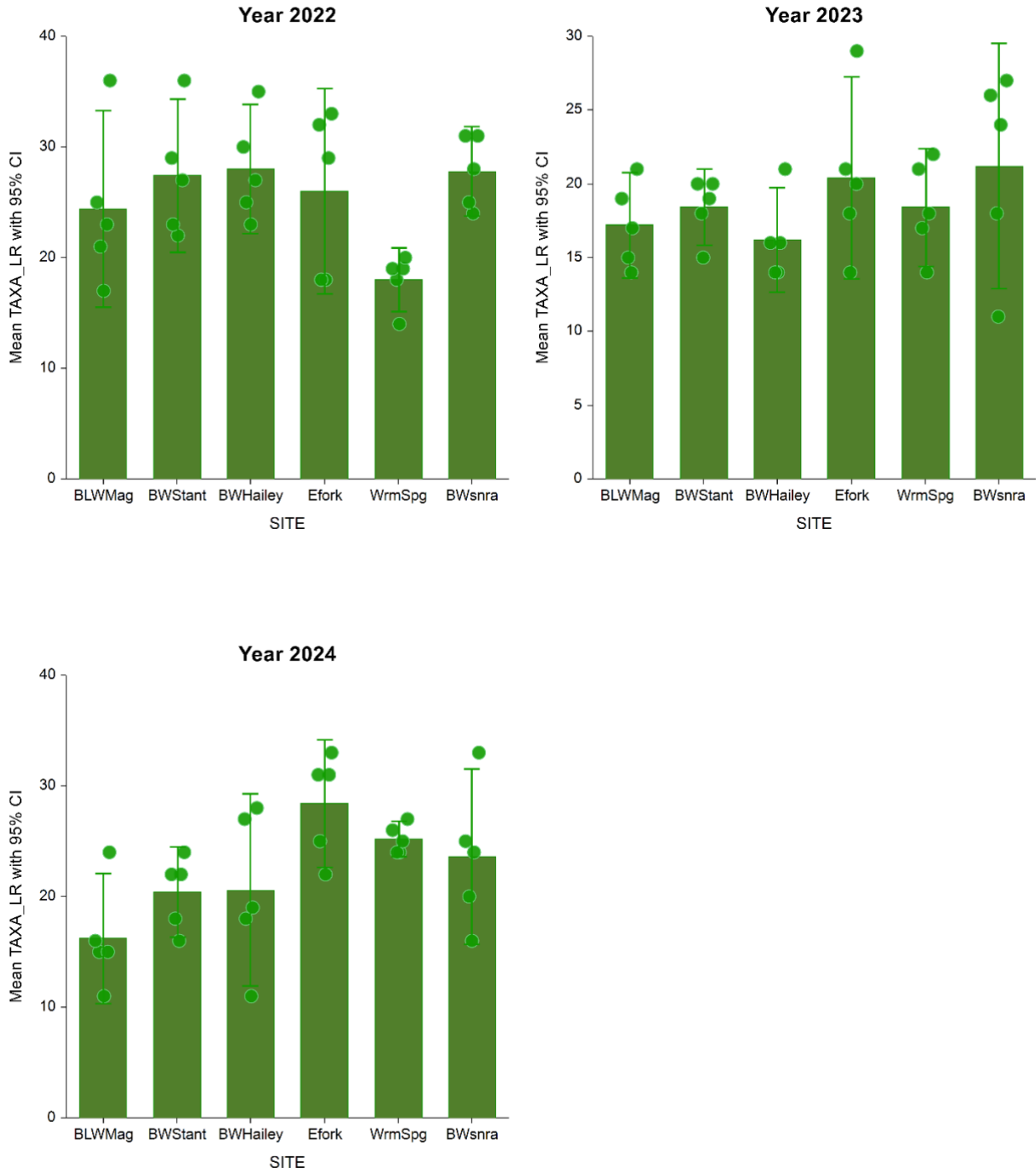
In 2024 the greatest Total taxa richness was observed at EFORK and the lowest occurred at BLWMAG. These were the only sites that appeared to have significantly different Total taxa richness values in 2024. Due to the high variation in observations, it is difficult to define spatial trends in 2024, but the sites that appeared to have somewhat lower richness occurred lower in the watershed. Recall from the previous section that BLWMAG declined in total richness each year.

Over the entire monitoring period there was no repeating pattern in the differences among sites—reflecting that individual changes at specific sites overshadowed any large regional shifts basin-wide. However, the lower Total taxa richness across all sites in 2023 might reflect an increase in the importance of regional conditions (e.g., climate) during that year. Recall that samples were collected early in the year each year, so invertebrate communities are reflective of conditions from the previous year. The most consistent “trend” among the sites was that EFORK and BWNSRA were usually among the sites with the greatest richness for the term of the monitoring program—and other sites were inconsistent in rank.

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<sup>4</sup> Prioritize ecological restoration efforts or prioritize protections for rarer pristine environments.

Differences among the sites each year: Total taxa richness ↓



## DIFFERENCES AMONG THE SITES IN EPT RICHNESS

In 2022, EPT richness was lowest at BLWMAG and WARMSP. BWNSRA stood out as having the greatest average EPT richness ( $\bar{x}$  =16) but was not statistically significantly different from any site other than WARMSP (see discussion of changes over time at WARMSP). Otherwise, the sites had similar average EPT richness values in 2022, and a wide range of variances made it difficult to detect the relatively small differences among sites. Many individual samples were below the 9 EPT taxa threshold—but averages were only below it at WARMSP and BLWMAG.

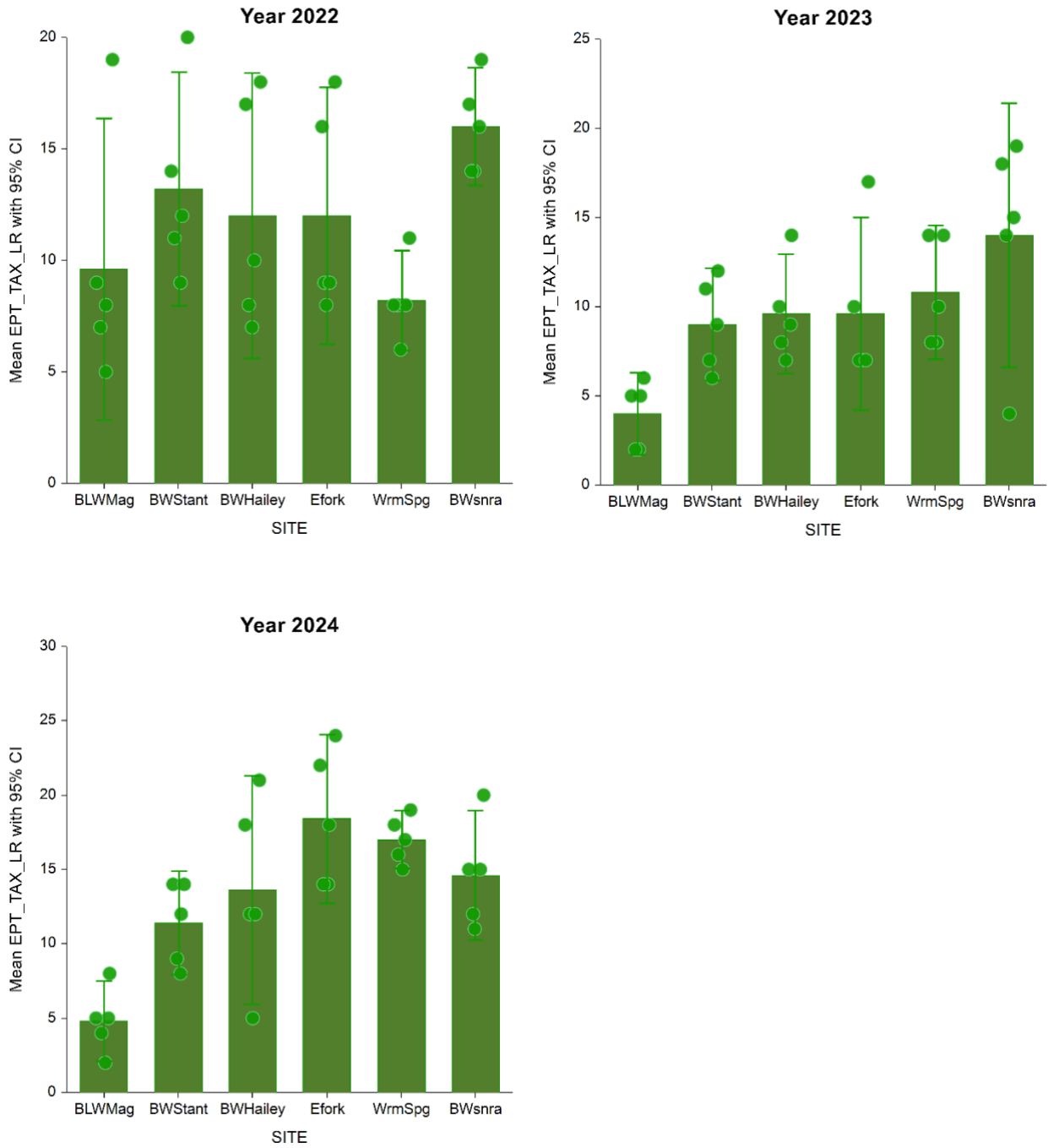
The differences in EPT richness among sites were more apparent in 2023, compared to 2022. The scale of the graph increased but this was driven by an increase in the individual values from BWNSRA—not by an increase in EPT richness across the board. BLWMAG communities exhibited a paltry average EPT richness below 5.0; most sites had average values around 9 and BWNSRA had greater EPT richness, but the mean was pulled down by one sample (q.v., BWNSRA changes over time in previous section). The average EPT richness basin wide was only 9.5 taxa.

In 2024, the pattern of differences among EPT richness values became more apparent—other sites increased and the average basin-wide EPT richness increased to 13.3 taxa. The greatest average EPT richness occurred at EFORK, WARMSP, and BWNSRA.

There appeared to be a similar spatial trend among EPT richness values among the sites in 2023 and 2024. EPT richness was generally lower at sites lower in the basin, and higher at sites higher in the basin. This “pattern” became more apparent in later years. BLWMAG seems to generally have lower EPT richness than the other sites—it was consistently below 10 EPT taxa on average for the entire monitoring period (with the exception of one sample in 2022) and averaged less than 5 taxa.



Differences among the sites each year: EPT richness ↓



## DIFFERENCES AMONG THE SITES IN EPT ABUNDANCE (%)

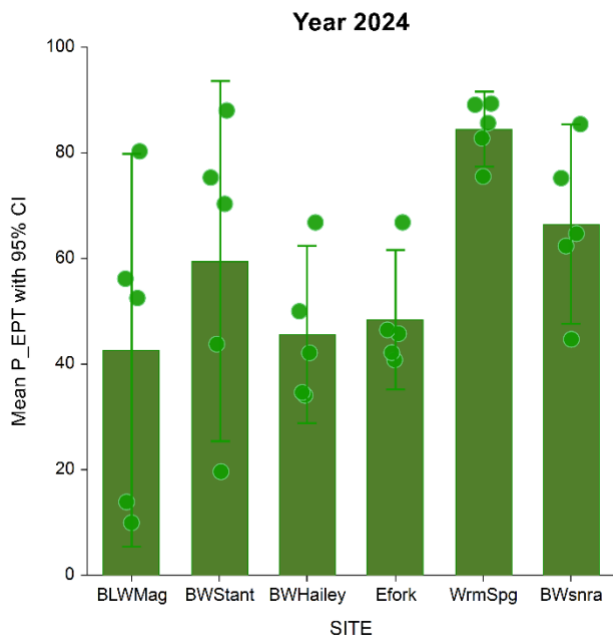
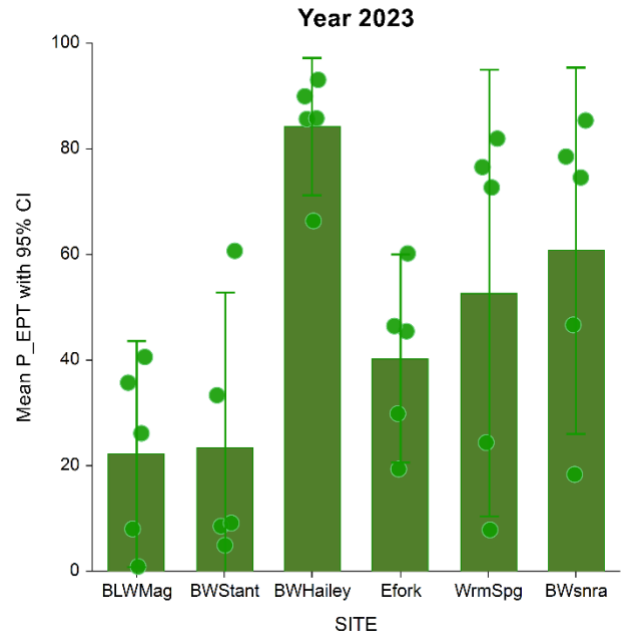
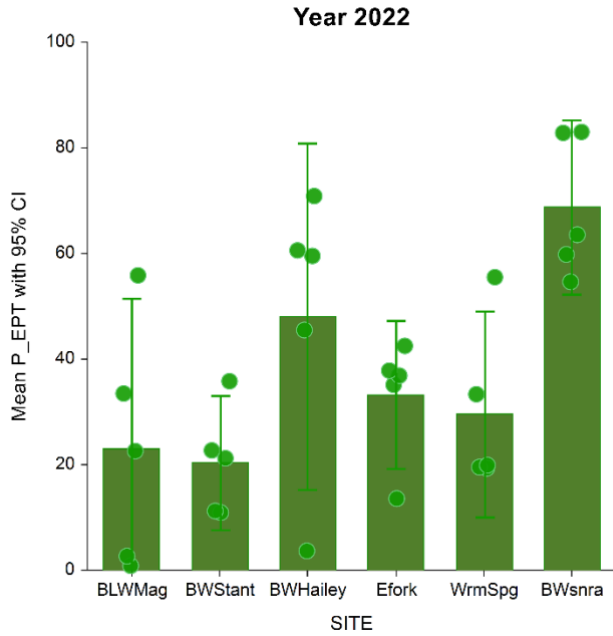
In 2022, EPT relative abundances varied widely among sites and within sites. Values were abnormally low at BLWMAG and BWSTANT. The greatest EPT abundance occurred at BWHAILY and BWNSRA. Wide ranges of variances among sites indicated that none of the differences among sites were statistically significant.

In 2023, average EPT relative abundances were abnormally low at BLWMAG and at BWSTANT. The greatest average EPT abundances were observed at BWHAILY and BWNSRA. Wide ranges of variance among and within sites indicated that the differences among most sites were not statistically significant. However, the site with the greatest EPT abundances (BWHAILY) was statistically significantly different from the sites with low EPT relative abundances (BLWMAG, BWSTANT, EFORK).

In 2024, the average EPT relative abundances were greatest at BWNSRA and WARMSP. The lowest observations were from BWHAILY, BLWMAG, and EFORK. However, the inclusion of BWHAILY and EFORK among the lowest sites was largely due to an increase in EPT abundance at BLWMAG and BWSTANT—not due to reductions in abundances at EFORK and BWHAILY. The wide range of variances among and within sites indicated that the differences among most sites were not statistically significant. However, the EPT abundance at WARMSP (the greatest %EPT) was significantly greater than EFORK and BWHAILY. This is more reflective of changes at WARMSP over time (q.v., changes at WARMSP).

The most notable trend in EPT relative abundance was that BLWMAG and BWSTANT had consistently fewer EPT's than most other locations. Although the mean values were higher at both sites in 2024, both sites still had some samples with only about 20% EPT. Additionally, BWNSRA had consistently high average EPT relative abundance during the entire monitoring period.

Differences among the sites each year: EPT Relative Abundance (%) ↓



## DIFFERENCES AMONG THE SITES IN MIDGE (CHIRONOMIDAE) ABUNDANCE (%)

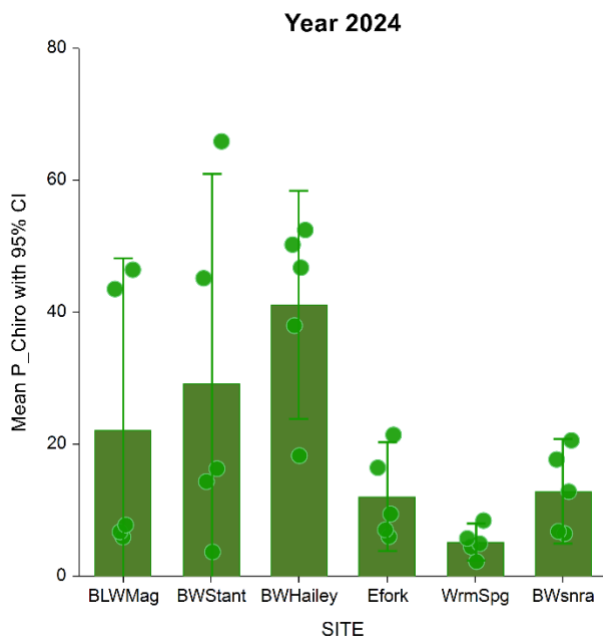
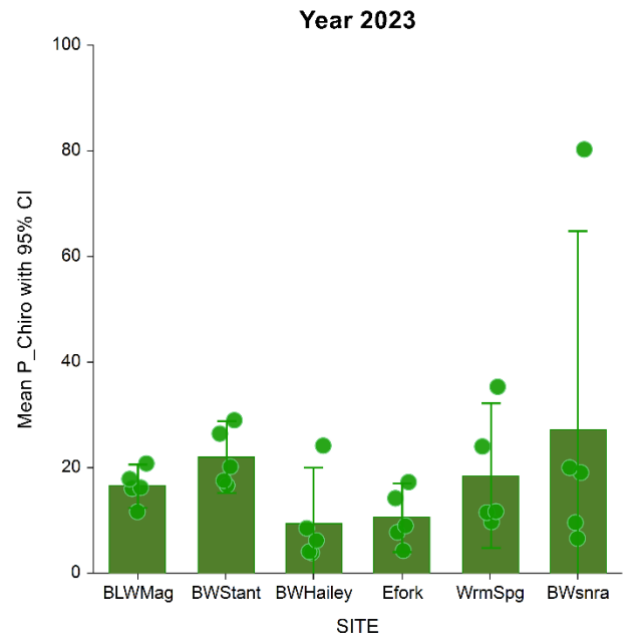
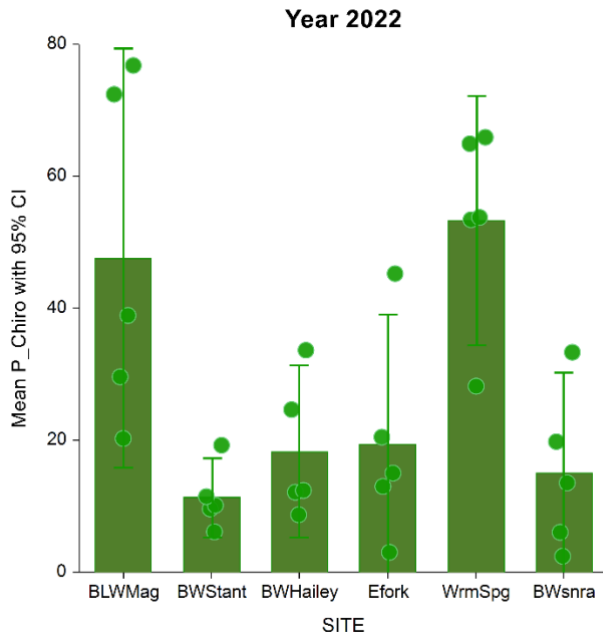
In 2022, the relative abundance of midges was variable. Although most sites showed variation in chironomid midge abundances, two sites stood out as having abnormally high chironomid midge abundances. Both BLWMAG and WARMSP had high midge abundance in 2023. One of the differences among sites was significantly different in 2022. Specifically, WARMSP exhibited statistically significantly greater chironomid midge abundances than BWSTANT. The benthic communities of most sites (BWSTANT, EFORK, BWSNRA) had normal contributions by the chironomid midges, except for the two high-midge sites.

In 2023, there were no statistically significant trends in the relative abundance of chironomid midges. Mean values were generally near or below 20% basin wide; this is ideal. One sample from BWNSRA pulled the average up slightly, but the average midge abundance and the observations from most individual samples collected from the site were consistent with observations basin wide and within the range of expectations.

In 2024 three sites (BLWMAG, BWSTANT, BWHAILEY) showed slightly elevated chironomid midge abundances compared to the three other sites (EFORK, WARMSP, BWNSRA). The only difference that was statistically significant was the difference between the site with the greatest midge abundance (BWHAILEY) and the three sites with the lowest midge abundance (EFORK, WARMSP, BWNSRA). However, the magnitude of midge relative abundance estimates was generally in the normal range, with BWHAILEY having moderately higher than normal average midge abundance in 2024. Although the averages were “normal,” some samples from BLWMAG and BWSTANT also had abnormally high midge abundance.

Generally, spatial patterns of chironomid midge relative abundances were more responsive to changes within sites over time (previous section on changes at sites) than they were to spatial patterns. That is, there were differences among sites each year (2023 notwithstanding) and the differences among sites were different each year. Thus, the factors affecting the spatial patterns of dominance by midges in the basin seem to be driven more by local conditions than by larger-scale regional phenomena.

Differences among the sites each year: Chironomid Midge Relative Abundance (%) ↓



## DIFFERENCES AMONG THE SITES IN NON-INSECT ABUNDANCE (%)

In 2022, high non-insect relative abundance occurred at BWSTANT and BWHAILEY. Average abundances of non-insects did not exceed 20% at the other sites, but some samples from most other sites were higher.

In 2023, average non-insect abundances in excess of 20% occurred at BLWMAG, BWSTANT, and WARMSP. Moreover, BLWMAG and BWSTANT exceeded this threshold greatly. The other sites had lower non-insect abundance. BWHAILEY and BWNSRA had ideal non-insect abundances.

In 2024, only BLWMAG exceeded 20% non-insects. BWSTANT, BWHAILEY, WARMSP, and EFORK all had very low contributions of non-insects to their assemblages. BWNSRA had greater variation in 2024 and had two samples with higher non-insect abundance.

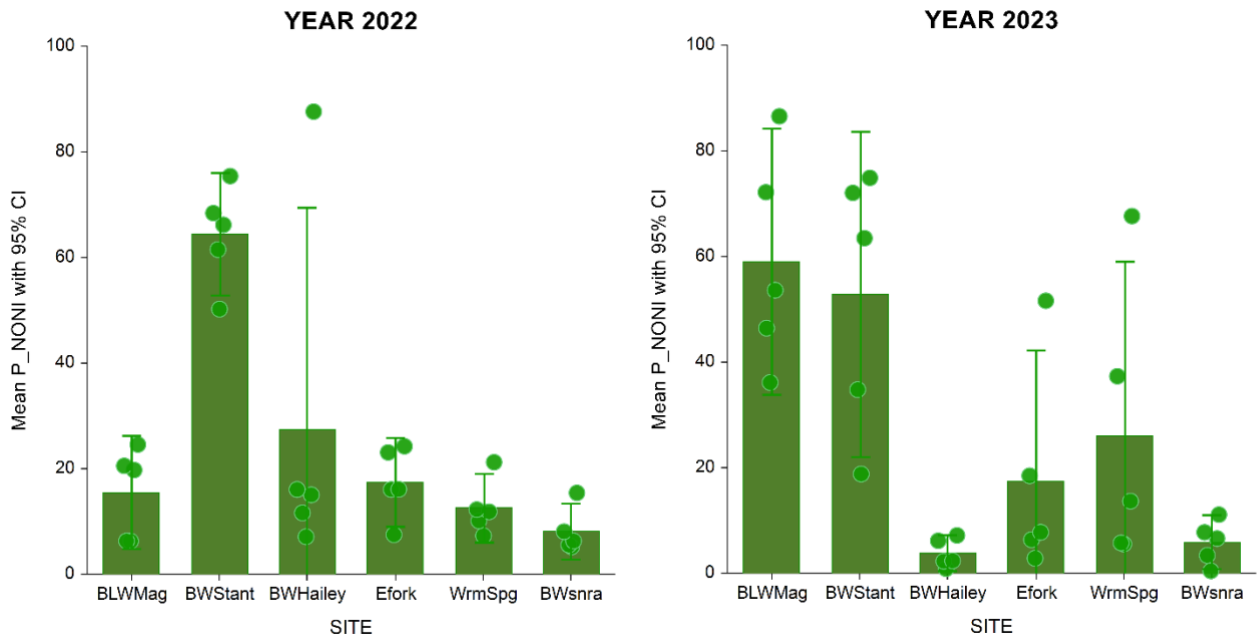
At first glance, it is difficult to resolve longitudinal patterns among the non-insect data that would constitute consistent spatial trends among the sites for the entire term of the monitoring program. A threshold was defined for the regional expectation of non-insects to comprise <20% (<15% ideally). Generalizing about this highly variable metric may appear abstract because the sites ranked<sup>5</sup> differently every year. Although most sites exceeded average non-insect abundance of 20% at least once during the monitoring period. EFORK and BWNSRA were the two exceptions to this observation, but even these sites had a few samples with higher abundances of non-insects.

Summarizing the frequency of exceeding this threshold shows a different kind of trend. (q.v., Supplemental figure below). Sites higher in the continuum of the basin generally had fewer occurrences of high non-insect abundances. Thus, although there was no single repeating pattern of abundances among the site's year-after-year, there was a longitudinal pattern of frequency of occurrence of high non-insect abundances. This method will be revisited in the summary of differences among sites.

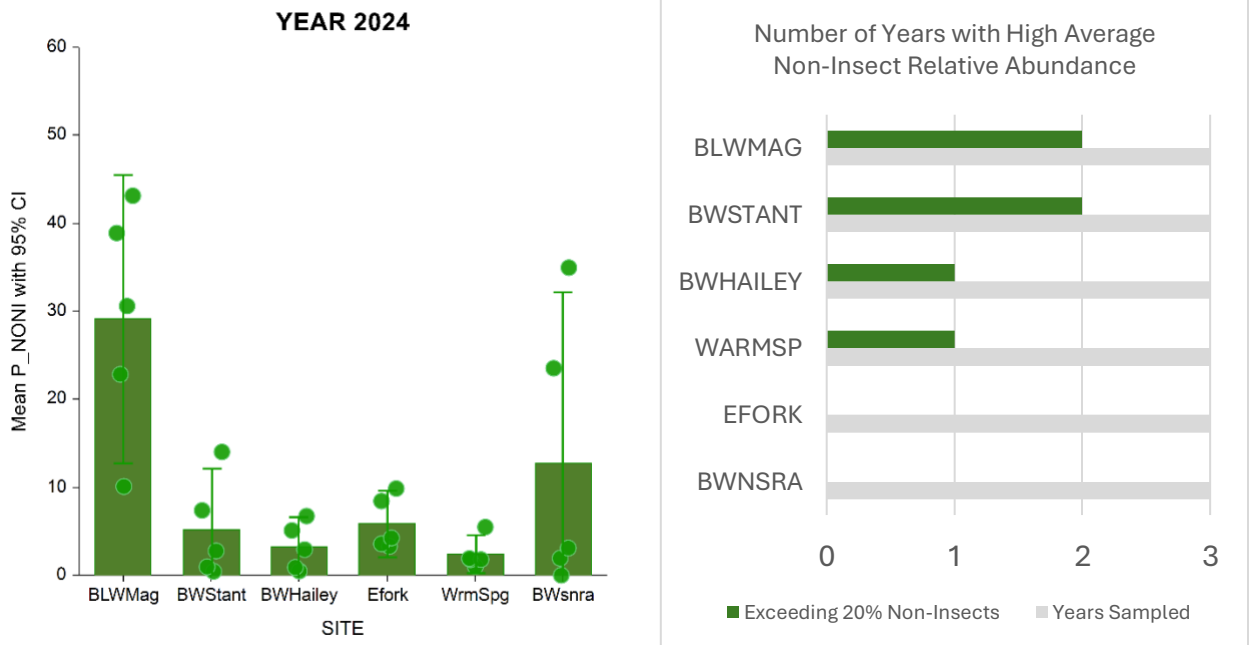
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<sup>5</sup> Ranks are determined by organizing observations in order of increasing magnitude for comparative or statistical purposes.

Differences among the sites each year: Non-Insect Relative Abundance (%) ↓



Supplemental Figure ↓



## DIFFERENCES AMONG THE SITES IN HILSENHOFF BIOTIC INDEX

As noted in the section focused on temporal trends at each site, the HBI primarily responded to changes in the relative abundances of EPT's, chironomid midges, and non-insects. Thus, it makes sense to use the HBI as a summary variable integrating the net effect of these changes. This is useful because in some years, non-insects displaced midges and EPT's, whereas in other years midges displaced non-insects and EPTs. Discussing the HBI's integration of these changes into a mean abundance tolerance of these groups is sometimes more useful than discussing off-setting changes in all the different groups of species. However, the other metrics provide context to this integrative variable.

To understand the response in this variable, it is important to understand how different groups contribute to increases and declines in HBI. In this study, midges were only identified to family—they were all Chironomidae. At the family level, chironomids have a collective HBI tolerance of 6.0. Thus, a sample that is 100% midges will have an HBI value of 6.0.<sup>6</sup> Similarly, most of the non-insects we identified in the Wood River Basin were tiny worms (*Nais* sp.) of the family Naididae. This group of small worms is given a tolerance value of 9.1 (Barbour et al. 1999<sup>7</sup>). Thus, a community comprised entirely of these worms would have an HBI value of 9.1. The occurrence of many midges in a sample will “pull” HBI values towards 6.0, whereas dominance by Naididae will pull samples above 6.0. Values that are significantly below 6 have communities with higher abundances of less-tolerant species, such as EPTs.

Because of this integration of all the off-setting changes in different taxonomic groups, longitudinal changes among sites were more apparent than they were with other summary metrics. Specifically, sites lower in the watershed had higher HBI scores, reflecting greater dominance of more tolerant species.

The sites with HBI values exceeding or near 6.0 were BLWMAG and BWSTANT. However, these sites exhibited a reduction in HBI in 2024—becoming more similar to the other sites. This constitutes an improvement in the community that is consistent with improving water quality. This may have occurred because improving regional conditions were able to overshadow local stressors that affected benthic communities sampled in 2022 and 2023 (e.g., temperature or discharge). Since samples were collected in the spring of 2024, this would reflect environmental conditions before April 2024 (perhaps April 2023-March 2024).

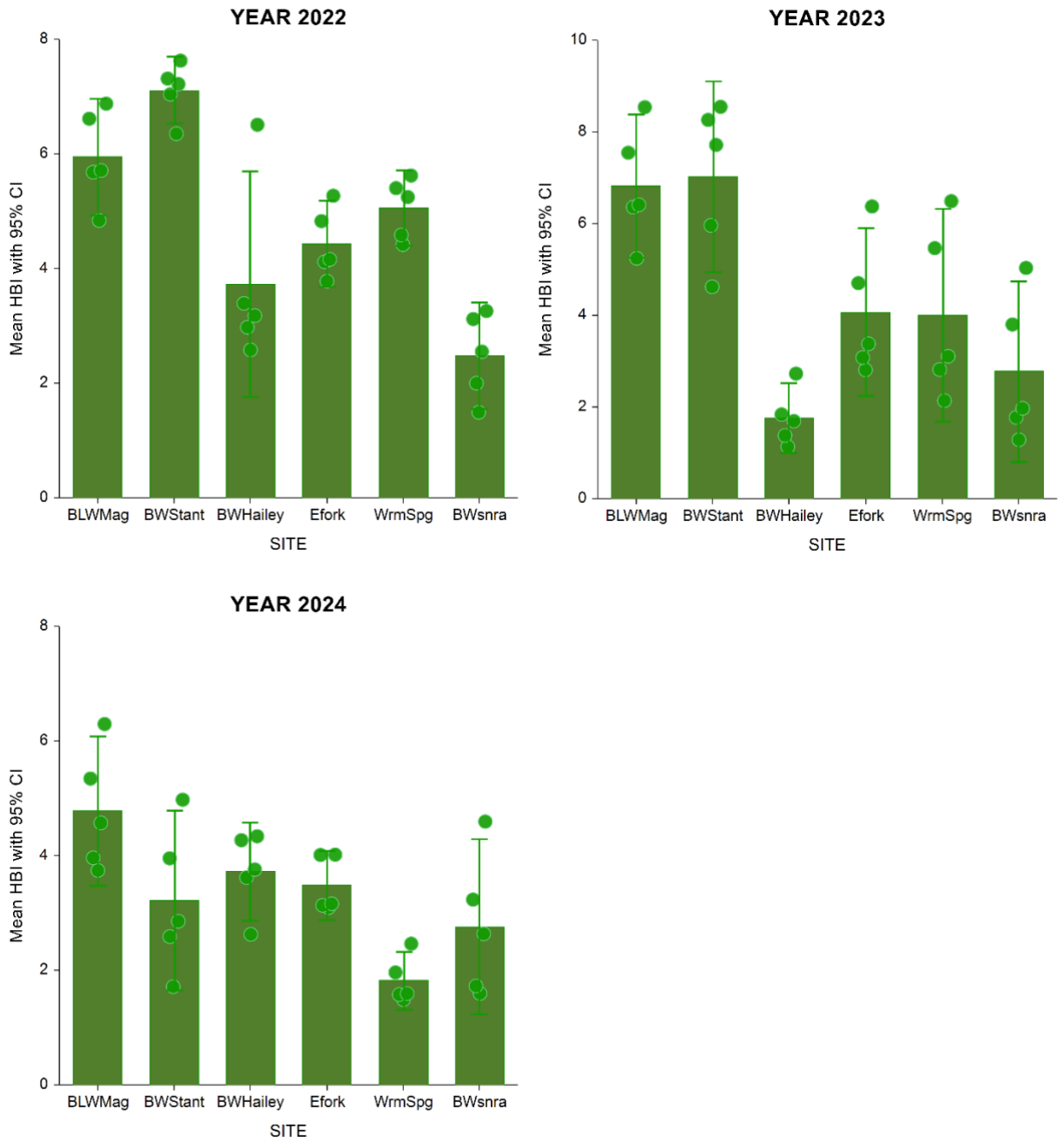
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<sup>6</sup> Although the family Chironomidae is given a value of 6.0, when midges are identified to greater resolution they may span the entire spectrum of tolerance scores and functional feeding groups.

<sup>7</sup> This is an EPA document. Hilsenhoff (1987, 1988) focused on insects, not-non insects. Non-insects were included in this document later.



Differences among the sites each year: Hilsenhoff Biotic Index (HBI) ↓



## SUMMARY OF LONGITUDINAL CHANGES IN BENTHIC ASSEMBLAGES

In this section, the method used to describe the longitudinal changes in the frequency of high non-insect relative abundances is revisited for all metrics. The thresholds used are based on experience of monitoring rivers in the area. Their scale is somewhat arbitrary, but once more data are amassed, watershed-specific criteria can be developed. Although there is a certain element of arbitrariness since these thresholds were not specifically developed for the Wood River Basin, they are nonetheless, based on experiences on other regional rivers (including the Henry's Fork River and the Big Hole River—among others).

Total taxa richness was excluded from this discussion because midges were only identified to family-level and most assessments we do involve identifying midges to the genus/species-group level. Identifying midges further usually adds 15-25 taxa to samples processed this way. Data used to define a regional threshold would require an assumption of the usual number of midge taxa in the Wood River Basin, which is beyond the scope of this report.

The regional EPT richness threshold of 9.0 is a reference drawing from a few sources, including experience in the intermountain west, but also formalized in some IBIs. Nonetheless, it can always be worse. Work in urban streams found most locations had zero EPT taxa, some had one or two taxa. And streams with three were considered good—and 4-5 were exceptional.

The threshold used for the EPT Relative Abundance metric is screening for average observations < 25%. Ideally, I like to see EPT abundances comprising 40-60%<sup>8</sup> of the community. But there is lots of variation around this “window” of ideal EPT-richness. Nonetheless, observations <20-25% would be considered low in the region—but not necessarily indicative of impairment.

Midges often comprise 50% of benthic assemblages. In coastal rivers, they may naturally comprise 60% or more of the community. However, observations from the area should be less than about 30-45% of the community. The threshold used here was that values > 30% constitute high midge abundance because it constitutes more than about 1/3 of the community, but this is not a hard rule for impairment.

Non-insects usually comprise less than 20% of the community. Lower values are better.

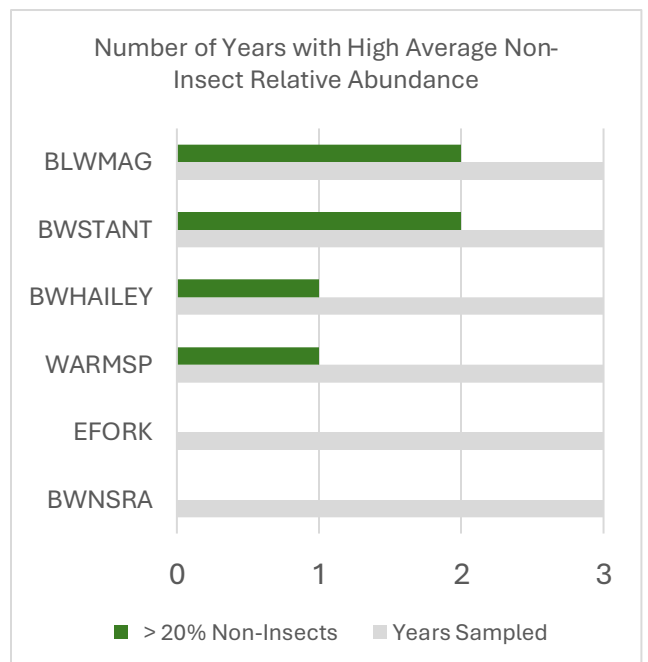
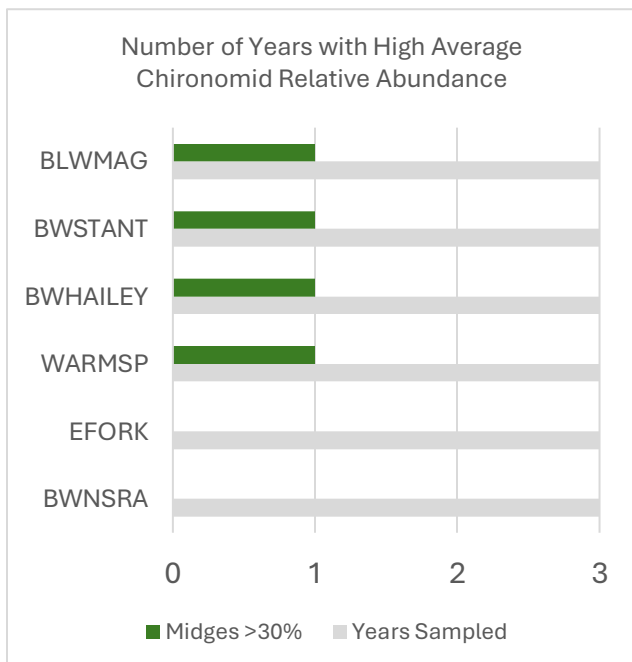
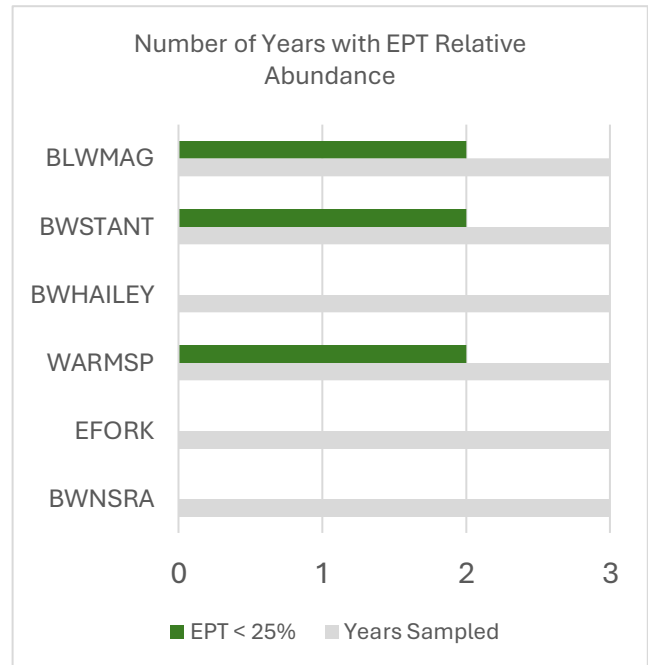
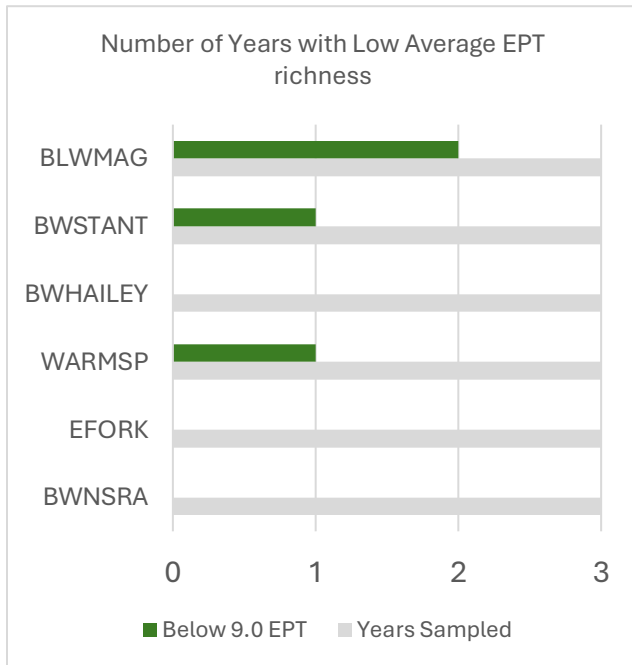
The HBI threshold for discussion in this section is HBI > 4.5. This is the middle of Hilsenhoff's (1988) “Good” category. Recall the scale of his measures in the methods section of this report. An intermountain community comprised 50% of very tolerant species and 50% very sensitive species is probably not a healthy ecosystem.

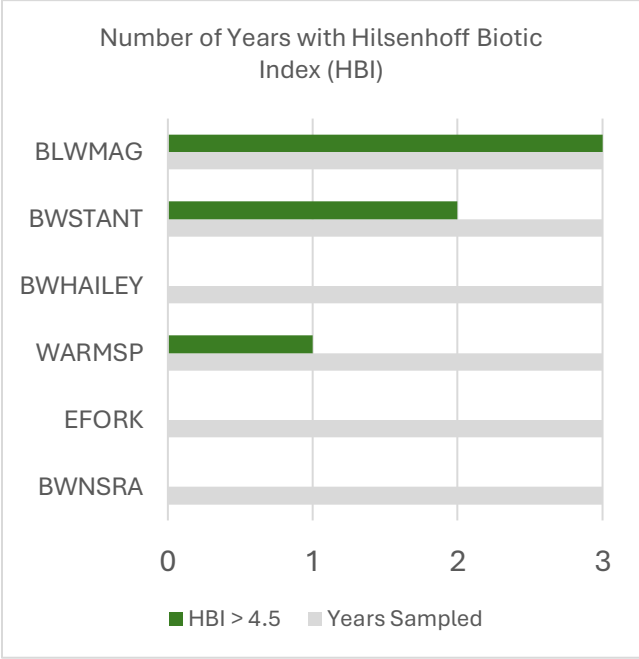
BLWMAG (10 deviations) was the site with the most frequent indications (“deviations”) of community stress for all metrics, with BWSTANT (8 deviations) often responding similarly. WARMSP (6 deviations) was intermediately deviant. BWHALEY had 2 deviations from expectations. EFORK and BWNSRA both had zero deviations from normal regional streams (Supplemental Figures, below).

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<sup>8</sup> Values near 80% often occur when samples are collected from either recently inundated stream channels—Baetid swimming larvae are very rapid colonizers. Alternatively, some dams cause exceptionally high densities of filter-feeding caddisflies and have very high EPT abundances. However, this was not the case for the observations here (q.v., WARMSP changes over time).

Supplemental Figures. The number of years (frequency) of each site deviating from the expectations of “normal” stream in the region.





## DISCUSSION

The changes at individual sites are discussed in detail above and summarized in bulleted lists in the Executive Summary at the beginning of this report. For the sake of brevity, these will not be repeated here. Although the analysis of different metrics defined few spatial trends that held up across all years, the longitudinal examination of metrics highlighted several sites that appeared more prone to disturbance than the others. Specifically, BLWMAG and BWSTANT had benthic communities that had metric responses indicating deviations from expectations more frequently than the others. EFORK and BWNSRA had no deviations from expectations using the thresholds defined in this report. However, these thresholds were not specifically developed to assess these sites.

Changes at individual sites were difficult to elucidate. This is because three years of data is often insufficient for trends to manifest. Additionally, the wide range of variances in most metrics made statistical resolution of differences in metrics difficult. There are several ways to reduce the impacts of this issue. First, the collection of additional replicates automatically narrows confidence intervals somewhat and increases statistical power. However, precautions have been taken in the lab to reduce certain forms of artificial variance inflation, and the current level of replication should provide a reasonable sensitivity to detect changes in richness. Some of the response signatures appear to show the same pattern of within-site dispersion for several different streams on the same day. This might suggest that the stratification parameters in use for the Wood River Basin are too broad. We have several strategies to control and statistically account for this variation and account for it statistically. This also increases the explanatory capacity of the monitoring program. However, these do increase the time it takes to collect samples in the field by about 30-50% per site. The specific method of sample collection and use of covariates will depend on the needs of the WRLT. For example, one of the best variables to stratify sample collection and statistically account for variation is sampling a narrow, but consistent range of water velocities at the water-substratum interface. This can (1) allow us to statistically adjust metric means for the influence of the pervasive effects of flow, and (2) compare differences among sites (or among dates) both before and after accounting for the influence of velocity. The approach to using this information depends on the importance of flow to questions asked.

Limitations to the study design aside, the monitoring program was clearly able to identify sites more prone to ecological perturbation and those which are not often subjected to disturbance. We can build upon this as more data are collected and build a baseline reference for these sites. This can be used to develop quick screening criteria to allow WRLT to quickly screen data for improvements relative to the established baseline. The utility and integrity of this process can be improved by comparing average metric values to reference criteria that are developed from 5-7 years of data using the mean of means to describe baseline conditions at the start of the monitoring period. This will enable WRLT to quickly screen and disseminate easy to digest annual summaries of changes in condition of the water resources monitored.

Although the study design can be refined, it is effective. There is even more information to be gained by examining this data set more completely. Changes in the relative abundance or density of dominant taxa (except for the changes in mayfly density at WARMSP) were not examined. In the section on HBI changes across the basin, we discussed how accounting for off-setting changes in taxonomic groups are aided by integration variables (like HBI). The same problem arises for examining changes in the abundance or density of all species—only it is much more complicated because it is a larger matrix (the matrix for this report would be about 9000 abundance estimates, many of them zeros). A tool to address this is multivariate statistical analysis, specifically Non-Metric Multidimensional Scaling. This provides a few 2-dimensional solutions

that best describe changes in all of our 100+ dimension starting model. These statistical methods do a great job of integrating the collective changes in the abundance of all species and even providing insight as to the condition of the stream bed (silt, sand, cobble, filamentous algae, etc.). However, they are not directly applicable to hypothesis testing (e.g., has Site-A changed between Year-1 and Year-2?). These methods are best used as part of an analytical approach describing changes in the river system several ways.

If this report is to be used to triage the sites that might benefit most from restoration efforts, consider BLWMAG and BWSTANT because they seem most prone to ecological perturbation. If the WRLT is interested in prioritizing conservation, then our results suggest that EFORK and BWNSRA might be the best sites to focus on because they were the least prone to ecological perturbation. WARMSP is an interesting community with an intermediate level of disturbance frequency. The re-establishment of high densities of mayflies at WARMSP in 2023 and 2024 is particularly interesting and indicative of improving condition. Changes in specific species might explain why and how the ecology at the site is changing.

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